FAT PARTITIONING AND SUBCLINICAL CARDIOVASCULAR

DISEASE AMONG WOMEN IN MENOPAUSAL TRANSITION

by

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In honor of my parents Bishwonath
and
Subhadra Devi Dhakal

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Obesity is one of the major risk factors of atherosclerosis and arterial stiffness. Recent evidence suggests detrimental effect of fat mass rather than overall body mass. Abdominal fat has been indicated to have more negative impact than other fat depots. We evaluated the impact of regional fat distribution on atherosclerosis and compared the variances explained by 11-different adiposity measures on atherosclerosis and arterial stiffness among bi-racial women in menopausal transition. All analyses were cross-sectional.

In the first analysis, adjusted for age, race, menopausal status, insulin, systolic blood pressure (SBP), triglycerides, height, high-density lipoprotein (HDL) and smoking; proportions of total (p= 0.03) and trunk fats (p= 0.03) were positively associated with common carotid adventitial diameter (AD). In contrast, proportion of leg fat was negatively associated with AD (p= 0.03). SBP attenuated the significant associations of total and regional fat distribution with carotid IMT.

In the second analysis, adjusted for age, race, menopausal status, height, SBP, low-density lipoprotein (LDL), HDL and insulin; waist circumference (WC) explained 25.2% of variance in IMT and 27.0% of variance in AD, while proportion of trunk fat

explained 22.7% of variance in IMT and 25.1% of variance in AD, and area of visceral adipose tissue (VAT) explained 22.7% of variance in IMT and 25.8% of variance in AD.

When adjusted for age, race, menopausal status, height, SBP, insulin and C-reactive protein; WC, proportion of trunk fat and VAT explained comparable proportions of the variance in carotid-femoral pulse wave velocity (cfPWV) (WC, 9.0% of variance; proportion of trunk fat, 9.9%; and VAT, 10.3%). After adjusting for above mentioned variables, only proportion of total fat remained positively associated with cfPWV (p= 0.04).

Overall, our findings provide evidence for differential role of regional fat distribution on atherosclerosis but not on arterial stiffness. Moreover, WC seems to be as good as computed tomography (CT) and dual-energy x-ray absorptiometry (DXA) measures of fat in explaining variability on atherosclerosis and arterial stiffness. Given the cost, difficulty in maintenance and exposure to radiation associated with CT and DXA, the use of WC in future research may have great public health significance.

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1.0   Dissertation Overview and Objective

Increasing prevalence of obesity has been constantly reported from different parts of world and has been considered an epidemic by the World Health Organization (WHO). At present, more than one billion people are overweight and 300 million of them are clinically obese [1]. In 2003-2004, 32.2% of adult Americans were obese [2]. Prevalence of obesity varies substantially by race, gender, age, geography etc.

Recent findings suggest that fat deposition in different parts of the human body may act differentially inducing adverse health outcomes. A negative role of visceral fat on the onset of chronic diseases has been highlighted by several reports [3-5]. Interestingly, some reports suggest that fat in lower extremities may be beneficial [6-8]. Thus, precise measurement of fat distribution in human body may be important in clinical evaluation of morbid obesity so that appropriate intervention can be implemented to curb negative impacts of obesity. Commonly used anthropometric measures may not be precise enough to determine the true picture of morbid obesity in real life. With technological development, several new methods including, bio-impedance (BIA), magnetic resonance imaging (MRI), dual-energy X-ray absorptiometry (DEXA) and computed tomography (CT) have emerged that are able to distinguish fat from lean and bone tissues and to estimate region-specific fat distribution.

Available evidence indicates that obesity is directly and/or indirectly associated with increasing risks of chronic disease including diabetes, insulin resistance, coronary heart disease, stroke, physical disability, dyslipidemia, hypertension, osteoarthritis, gallbladder disease, sleep apnea, respiratory problems and some cancers [9]. Currently, at least 34.2% of Americans have one or more risk factors of CVD [10]. Evidence indicates that prevalence of CVD is higher among males than females until they reach in early-50s however, it is just the reverse there after. One

plausible reason for higher prevalence of CVD among women after the age 50 is vital changes in hormonal flow in their body as a result of menopause which occurs around mid-forties. Prior studies have found significant increase in abdominal obesity and CVD prevalence among post-menopausal compared to pre-menopausal women [11]. However, data on obesity and CVD during menopausal transition is still scare.

This dissertation is planned to investigate changes in abdominal fat deposition and their impact on subclinical markers of CVD. This dissertation will attempt to address following research hypotheses;

I.   Higher abdominal fat is associated with increase in carotid intima-media thickness (IMT) and carotid adventitial diameter (AD),

II. The variances on carotid IMT and AD by abdominal adiposity measured by waist circumference (anthropometry), trunk fat (DXA) and visceral adipose tissue (CT) are comparable and,

III. Visceral adipose tissue (measured by CT) explains more variance on arterial stiffness (measured by pulse-wave velocity) than waist circumference or trunk fat (measured by DXA).

2.0   Background

2.1   Obesity

2.1.1   Epidemiology of Obesity

Ever increasing obesity across the world has been recognized as one of the most important public health concerns today. While the number of deaths from under-nourishment is declining, the number of deaths attributed to obesity is at an all time high and is still increasing. Worldwide, more than one billion adults are overweight and at least 300 millions are clinically obese. In 2003, the highest proportions of obese people were in urban Samoa (75%) and the lowest (approx. 5%) in China, Japan and some African nations [1].

Today, two in three Americans are either overweight or obese. In 2003-2004, 32.2% US adults (20 years of age or older) were obese (defined by body mass index (BMI)≥ 30 kilogram of weight/meter squared of height). Evidence suggests that prevalence of obesity varies widely across geographical regions, ethnic background, co-morbid conditions, gender and age [12]. Obesity in the US was found to be more common among non-Hispanic African Americans (45.0%) followed by Mexican- Americans (36.8%) and non-Hispanic Caucasians (30.6%) [2]. Fat accumulation among men and women varies considerably with natural aging (see figure 2-1). The highest prevalence of overweight and obesity in male starts early, after 35 years of age and then decreases slightly after the age of 75, whereas in female, the prevalence spikes after the age of 45 and decreases after the age of 65 [11]. These findings are partly explained by the change in hormonal flow at menopause are responsible for such variations, at least partly.
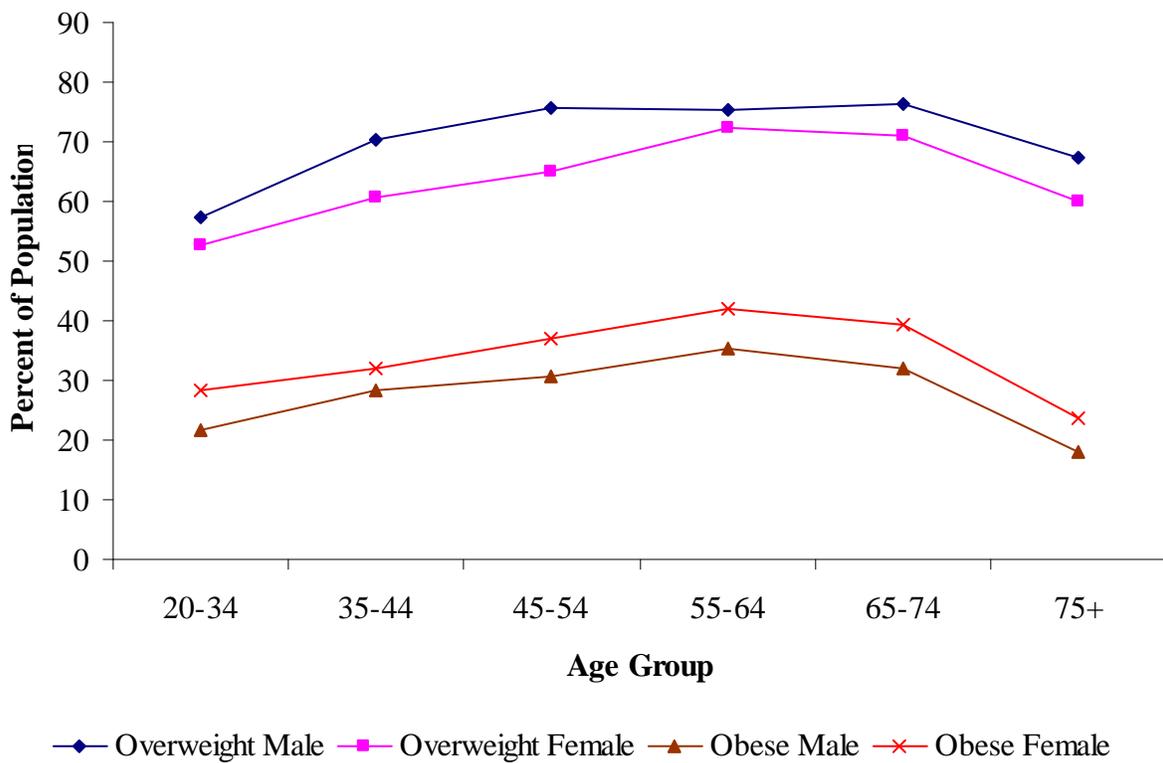
Figure 2-1 Prevalence of overweight and obesity in US by age and sex (1999 –
2002)

Recently, obesity has been increasing more rapidly than overweight. Proportion of
overweight American, aged 20-74 years, has increased by more than 45% in 1999-2002
compared to 1960-1962; whereas, for the same period and age group, obesity rate
increased by more than 133% (figure 2-2) [11].
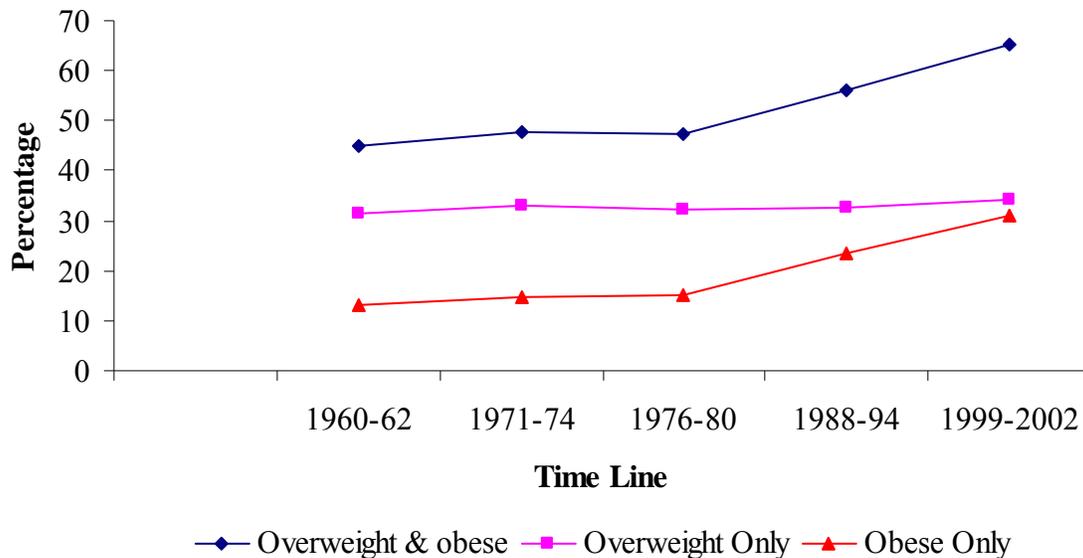
Figure 2-2 Overweight and obesity among American adults (1960-2002)

Obesity is a complex chronic condition that results from interaction of environmental, social, behavioral and genetic factors. It has been believed that genes make people susceptible to obesity [13,14], which upon interaction with other environmental, social and behavioral factors for a long time ultimately leads to overweight and finally obesity. However, the exact magnitude of genetic contribution in prevalence of obesity is yet to be identified.

From financial perspective, obesity induced negative health outcomes result in enormous amount of health care expenditures. According to Colditz et. al., direct cost of obesity (not overweight) in 1995 was 70 billions US dollars in the US, 7% of the US health care expenditures for the year [15]. The estimate included the diagnosis and treatment costs (hospital or nursing home stay, medications, physician visits) incurred because of type II diabetes, coronary heart disease, hypertension, gall bladder disease, osteoarthritis and, breast endometrium and colon cancers. The

cost of obesity and overweight in 1998 was estimated to be 78.5 billion; 9.1% of the total annual US medical expenditures [16].

2.1.2   Adipose Tissue Metabolism

Adipose tissue represents 15-18% of the body weight in males and about 25% in females under normal physiologic conditions [17]. Energy intake in excess to expenditure results in positive energy balance in the human body. Excess energy is stored, in the form of fat. Continuous increase in fat storage leads to obesity. It has been understood that adipocytes (fat cells) play a major metabolic role on food intake, energy balance, and metabolic homeostasis through endocrine hormones. The body receives fat either from dietary source (as lipid) or synthesizes from other nutrients including proteins and carbohydrates. Lipids in diet are available in the form of triglycerides, free fatty acids, cholesterols and other sterols. Also, excess proteins and carbohydrates in the body are metabolized by liver into lipids. Liver is primarily responsible for lipogenesis, whereas adipose tissue stores the lipid. Fat, first absorbed in small intestine are converted to triglyceride forming large fat globules with free fatty acids, phospholipids and sterols. These fat globules react with bile salts in intestinal lumen which are then hydrolyzed by pancreatic lipase and form monoglycerols and long chain free fatty acids. Monoacyglycerols are then converted to di- or tri- aciglycerols and cholesterol esters are formed from fatty acids.

Chylomicrons are covered by a shell of protein, cholesterol and lipoprotein allowing lipids inside to float freely in the water-based bloodstream. The main purpose of chylomicrons is to emulsify dietary fats before they enter bloodstream. Chylomicrons then travel to thoracic duct that opens into a large vein called subclavin vein via lymphatic system. After breaking down of triglycerides by lipoprotein lipase into free fatty acids and glycerols, majority of glycerols travel to cells in liver and kidney through blood and turned into glucose. Adipose, muscle and other

cells in the neighborhood area absorb most free fatty acids for immediate fuel or are re-esterified into triglycerides. Adipose cells tend to re-esterify and store fatty acids as triglycerides, whereas, muscle cells tend to de-esterify and metabolize them. After uptake of triglycerides from chylomicrons, the remnants that mostly contain cholesterol and protein are then taken up by liver for further metabolism. Unabsorbed free fatty acids bind to albumin for distribution in the circulation.

2.1.3   Assessment of Adiposity

There are several ways to quantify obesity including anthropometric measurements (BMI, waist circumference (WC), waist-to-hip ratio (WHR), triceps skin folds etc), DXA, BIA, MRI, CT etc [18,19]. Although not very precise, anthropometric measures are widely used in field research as markers of body fat because of their ease of use and cost-effectiveness. A historical overview of anthropometric indicators of abdominal obesity is given in table 2-1.

Table 2-1: Overview of adiposity measures

Indicator	Proposed by	Initial Reason to use	Merits	Demerits
Waist-Thigh Ratio (WTR)	Ashwell et al (1978, 1982)	To classify android vs gynoid obesity	Better correlate of visceral fat/ risk factors than WHR	- Reflects muscle & fat distribution, - not easy to interpret
Waist-Hip Ratio (WHR)	Krotkiewski et al (1983) Hartz et al (1983)	To classify android vs gynoid obesity	Good predictor of mortality & morbidity	- Reflects muscle & fat distribution, - not easy to interpret
Sagittal Abdominal Diameter (SAD)	Kvist et al (1987)	High correlation with visceral fat observed by CT scan	Best correlate of visceral fat	- Difficult to measure in field research than WC
Waist-Height Ratio	Higgings et al (1987)	Lack of measurements of hip and thigh	Better predictor of visceral fat than WC or WHR	- Height inversely associated with morbidity/ mortality independent of fat distribution
Conicity Index	Valdez (1991)	To standardize WC for body shape	- Built-in adjustment of WC for height & weight - Does not require hip circumference	- not easy to interpret
Abdominal diameter-Mid thigh girth Ratio	Kahn et al (1993)	Better predictor of IHD & mortality from sudden coronary death than WHR	Best simple index to predict morbidity and mortality	-Reflect both muscle and fat distribution - not easy to interpret
Waist Circumference	Pouliot et al (1994) Lean et al (1995)	-Correlation with visceral fat - Replacement of BMI and WHR	- easy to interpret - Better correlate of visceral fat than WHR and WTR	High correlation with BMI

BMI has been widely accepted as a marker of body weight relative to height in research and clinical practice. Table 2-2 describes the most commonly used cut-off levels of BMI to define under weight, healthy weight, over weight, and obesity in population. Anthropometric measures provide different information regarding the regional distribution of adiposity. WHR indicates upper versus lower adiposity, whereas WC more specifically reflects abdominal fat storage. Biological interpretation of WHR is not as straight as of WC, since the hip component incorporates fat and muscle mass at the gluteal level [20].

Table 2-2. Classification of overweight/ obesity by BMI and associated disease risk

BMI(kg/m2)	Weight Class	Obesity Class	Disease Risk[1]
<18.5	Underweight		-
18.5-24.9	Normal Weight		-
25.0-29.9	Overweight		Increased
30.0-34.9	Obese	I	High
35.0-39.9		II	Very High
>40.0	Extremely Obese	III	Extremely High

[1]- Disease risk for type 2 diabetes, hypertension and CVD

Although BMI is correlated with total fat mass and fat percent, it is not considered a precise indicator of underlying proportion of fat and lean tissues. Previous studies from Asia have reported that Asians have high visceral adipose tissue despite their normal range of BMI [21-23]. Similarly, Kunesova et al found WC and SAD as better predictors of metabolic variables and arterial blood pressure than WHR [24]. More accumulation of fat in the visceral region than other parts of the body may partly explain these associations. In another study, BMI identified obesity in only 50.6% of racially mixed population and there were 49.4% false-negative results

(inaccurately classified as lean) [25] indicating the need to redefine obesity on the basis of visceral and/ or total fat content rather than just BMI.

2.1.3.1 Dual Energy X-ray Absorptiometry and Computed Tomography

Although anthropometric measures are most commonly used markers of adiposity in clinical as well as research settings, they have limited ability to differentiate the contribution of fat and lean mass on disease outcomes. Hence, newer technologies DXA and CT that are able to differentiate fat and lean mass have been suggested as more precise and accurate in measuring the total and regional distribution of fat and lean mass and to estimate their contribution to disease outcomes [18,26]. There is a substantial need for an 'ideal' marker of overall and regional adiposity that; a.) is accurate in measuring adiposity, b.) can predict disease risk in general population, c.) is applicable and acceptable in large population studies as well as in clinical settings and d.) is precise resulting in small measurement error. However, a clear agreement on ideal marker of overall adiposity and regional adiposity is still far from reach.

DXA methodology is simple, suitable for general population including elderly and very sick people. Because of its ability to differentiate adipose tissue from lean and bone mass with low radiation and its better applicability in large population settings than CT and MRI, it is now considered the gold standard for body composition [18,26]. The basic concept of DXA technology is the energy attenuation in vivo is a function of tissue composition. This technique assumes that the body is composed of three components; fat, bone and lean tissue, which are distinguishable by respective energy attenuation properties reflecting their differences in densities and chemical compositions. The human body is divided into a series of pixels within which the energy attenuation is measured at two energy levels. Within each pixel, proportions of only two components can be solved by the differential absorption of two energies. With increasing energy

levels, the difference in the attenuation properties for tissue decreases. Image generated by the scan is first analyzed to calculate the bone threshold and identify bone and non-bone points. Attenuation of x-ray by pure fat and non-bone lean tissue is known and the analysis proceeds on a point by point basis. Each point is sub-divided into fractions of lean and fat mass.

CT is a noninvasive technique that uses X-rays in a fan-shaped beam detected by an array of detectors, available at the opposite side of a subject. CT, in addition to its ability to distinguish fat, lean and bone tissues has ability to identify visceral organs and to distinguish intra-abdominal fat from subcutaneous fat [18,27]. Like DXA, the basic concept of CT methodology relies on energy attenuation properties of different tissues. The intensity of the transmitted X-ray is detected by detectors providing information on bodily structures that the beam passed through [28]. Associated computer processes the cumulative information using available algorithm and produces cross-sectional image of the scanned area. These images can be used to distinguish visceral adipose tissues from subcutaneous adipose tissues. Radiation dose and associated cost have been its major disadvantage.

2.1.4 Regional Distribution of Adipose Tissue

Recent studies have highlighted that the differential impact of regional fat distribution may be more important than total fat on adverse health outcomes. Central adiposity, mainly assessed by anthropometric measurements including BMI, WC, WHR, is associated with cardiovascular morbidity and mortality [4,8,29-35]. Further more, android fat accumulation in women is associated with cardiovascular morbidity and mortality, independent of the degree of obesity [36]. A major limitation of anthropometric measurements is its' inability to distinguish fat from lean and bone tissues and differentiate fat stored in visceral organs from fat stored subcutaneously. Accumulation of VAT exerts more negative impacts on health outcomes than

subcutaneous fat [37-39]. VAT is thought to be the main adiposity marker associated with traditional CVD risk factors because of it's active pro-lipolytic activity and higher ability to deliver free fatty acids and inflammatory cytokines directly to systemic circulation than other fat depots[26]. Most importantly, free fatty acids released from VAT have direct access to liver which may impact negatively on hepatic insulin activity increasing the risk of diseases [9]. Prior studies have also shown an inverse relationship of circulating adiponectin with abdominal adiposity suggesting lowered anti-atherogenic activity of adiponectin [40-43] with increased abdominal adiposity.

It has been shown that visceral fat deposition varies by ethnicity, geography, culture, heredity, socio-economic factors, menopausal status etc [44]. The prominent role of visceral adipose tissue on health outcomes including, metabolic syndrome, insulin resistance, CVD have been consistently shown in recent studies [45-48]. On the other hand, some studies have identified a negative correlation between fat in lower extremities and glucose and lipid levels in postmenopausal women and elderly population [6,7]. Lower levels of atherogenicity with increased peripheral fat mass and leg fat percentage has also been reported [8,49,50]. This may be because the adipocytes in the lower extremities, particularly in the femoral-gluteal area are relatively sensitive to anti-lipolytic activity while being less sensitive to pro-lipolytic activity.

Studies examining the impact of menopause on fat distribution have found inconsistent results. Different techniques employed to measure body fat have been suggested as one potential explanation for such discrepancies [51]. A significant effect of menopause transition, independent of age and total adiposity, on body fat distribution has been reported consistently by prior studies that used DXA to determine abdominal adiposity [52] [53-55]. Studies using computed tomography have also found significant effect of menopause transition on body fat distribution even after

controlling for BMI or age [56,57]. Kotani et al observed an accelerated accumulation of visceral adipose tissue (VAT) with increasing age among postmenopausal women. In another study, after adjusting for age and total fat mass, postmenopausal women had significantly more intra-abdominal adipose tissue compared to pre-menopausal women[58]. Gambacciani et al., found increased abdominal fat accumulation, even during the perimenopausal period [59]. Studies using WHR or waist circumference to define abdominal obesity failed to find significant effect of menopause on body fat distribution after adjusting for potential confounders including degree of obesity, BMI and age [56,60-68].

2.2 Cardiovascular Disease

2.2.1 Epidemiology of Cardiovascular Disease

According to American Heart Association, over 71 million American adults were living with at least one type of CVD in 2003, while 37.3% of all deaths in the US were attributed to CVD in the same year [10]. Out of 910,614 CVD deaths in the US in 2003, 53.1% were females. Although proportion of people dying of cardiovascular disease has been decreasing in recent years compared to 1950, it is still the number one killer in the US. According to Center for Disease Control and Prevention (CDC), probability at birth of eventually dying from major CVD is 47% [69]. In general, CVD incidence and prevalence increases with age in both genders, the rate of increment is much higher among females after menopause (figure2-3). CVD also induces huge economic burden to the population. For 2006, CVD was expected to cost more than US $ 400 billions in direct and indirect cost [10].
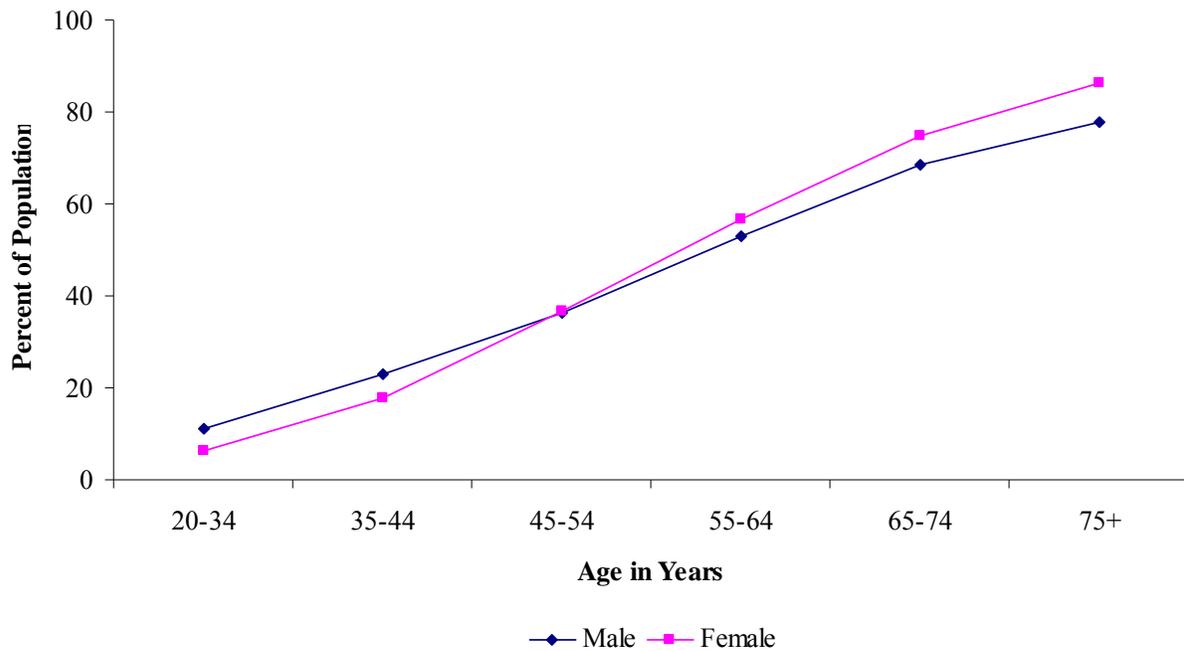
Figure 2-3: Prevalence of cardiovascular disease in USA by age and sex (1999 – 2002)

2.2.2 Subclinical Cardiovascular Disease

Atherosclerosis is the narrowing and hardening of arteries caused by the accumulation of cellular waste products, fatty substances, cholesterol and other substances within the intima-media layer of the artery [70]. Atherosclerosis is a slow, progressive disease that may start in childhood, although it does not become clinically apparent until young adulthood or later.

In atherosclerotic process, endothelial layer (an interface between blood and artery, figure 2-4) as weakens activates cell adhesion molecules that allow the attachment of leukocytes to endothelial wall [70,71]. Monocyte chemoattractant protein-1 (MCP-1) activates leukocytes to enter the intima and they begin to store lipids. Next, leukocytes transforms into a lipid loaded macrophages called foam cell [70] which is considered the first major lesion of the atherosclerotic process and is commonly called 'fatty streak'. By multiplication of smooth muscles cell that accumulate plaque, the lesion starts to grow forming a fibrous cap which causes to narrow lumen

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diameter ultimately limiting blood flow. Fibrous cap if ruptured, with the continuous influx of macrophages and inflammatory cells into the plaque may lead to thrombosis and occlusion of arteries leading to CVD events including myocardial infarction and stroke [72-74].
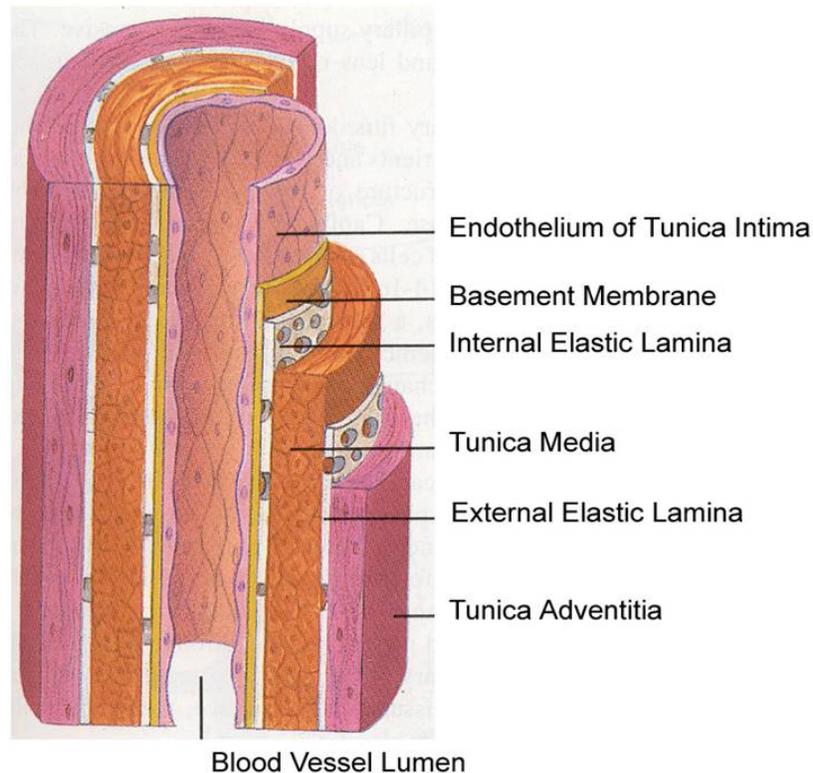
Figure 2-4. Schematic illustration of various layers of the arterial wall

(Source: http://bme.engr.ccny.cuny.edu/faculty/jtarbell/SMC%20images.htm)

Several methods of measuring subclinical atherosclerosis have been suggested in literature including intima-media thickness, adventitial diameter, ankle-brachial index and calcification [73,75-77]. Intima-media thickness and adventitial diameter of carotid artery were used as markers of subclinical atherosclerosis for the purpose of this dissertation.

2.2.2.1 Carotid Intima Media Thickness and Adventitial Diameter

Carotid atherosclerosis and atherosclerosis in the coronary and peripheral regions are positively correlated [73,78-80]. Moreover, carotid IMT is positively associated with several

established risk factors of atherosclerosis including age, weight, SBP, insulin, lipids, smoking, inflammation [81] and with prevalent and incident CHD[75,76,82,83]. The positive association between the multi-factorial Framingham risk score and carotid IMT also indicates that there is an additive effects of traditional CVD risk factors on IMT [84]. Because of high correlation of carotid arterial disease with disease of other arterial beds, ease to identify and non invasive in nature, it is widely accepted measure of subclinical atherosclerosis [77].

Carotid adventitial diameter, like carotid IMT, is correlated with established risk factors of atherosclerosis and, is positively associated with coronary disease and coronary disease syndrome[85-90]. Since change in arterial diameter occurs 'very early' in the atherosclerotic process, understanding the change may be helpful in predicting CVD events and thus may improve current understanding of atherosclerotic process[87,91-93].

Measurement of carotid Intima-media thickness and adventitial diameter are non-invasive, reliable, readily available and cost effective tools to measure subclinical burden of atherosclerosis. The B-mode ultrasound methodology, used in measuring IMT and AD, relies on acoustic resistance of various tissues and the resultant localization of signal at tissue boundaries. Collagenous and calcified tissues have very high relative acoustic independence (on the basis of tissue density) which provides clear delineation of the collagenous tunica adventitia of arteries, of collagenous atherosclerotic lesions and of the intima-media boundary. Images of right and left common carotid artery (CCA), common carotid bifurcation and the first centimeter of the internal carotid artery (ICA) as generated by sound waves are commonly used to measure IMT. CCA images are taken at diastole on upstroke of the R-wave while bulb and ICA images are taken from T-wave to the upstroke of R-wave. Later, an automated software Artery Measurement System (AMS) is used to score (for example, an average of the distance from

lumen-intima interface to media-adventitia interface in both walls, for common carotid artery) each image (figure 2-5). Use of automated software, improves intra- and inter reader variability, significantly reduces measurement time, is user friendly and the use of standard algorithm reduces the 'subjectivity'.
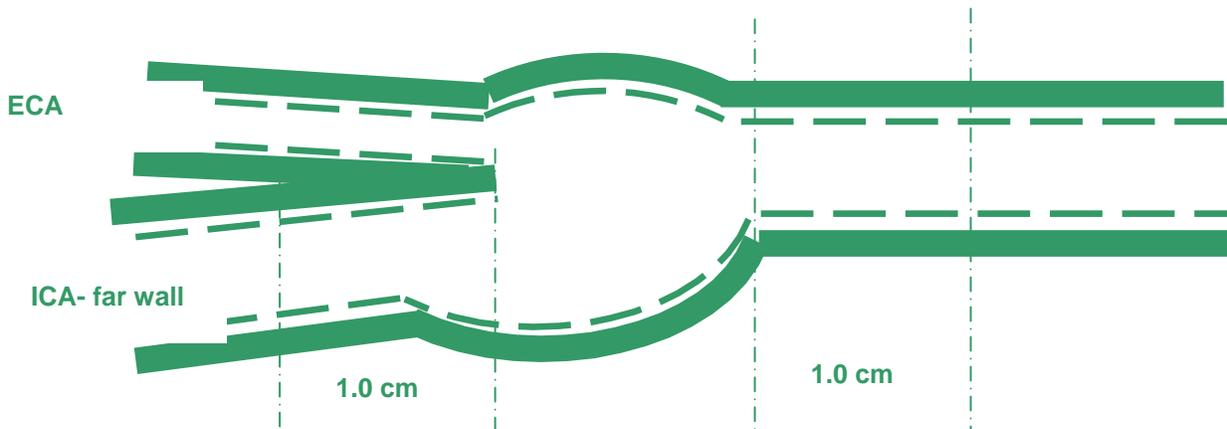
Figure 2-5 Schematic view of carotid artery

Tunica adventitia includes external elastic lamina, terminal nerve fibers and connective tissue containing fibroblasts and tissue macrophages. Vascular remodeling is more a function of plaque dynamics than location. In general, the expansive remodeling occurs first because of several reasons including hemorrhage into a plaque. While constrictive remodeling occurs because of frequent plaque rupture or healing process or accumulation of collagen [93,94]. Inflammation of adventitia is an early component of atherosclerotic vascular proliferation [93]. Also, recently it has been understood that cellular elements infiltrating the tunica media and intima in atherosclerosis are derived from adventitial myofibroblasts [95,96]. Adventitial diameter is calculated by adding lumen diameter to IMT of near and far walls of CCA.

2.2.2.2 Arterial Stiffness and Aortic Pulse Wave Velocity

Prior studies have found positive associations between central arterial stiffness, as measured by aPWV and, cardiovascular and total mortality [97-100]. In a cohort of healthy older adults, Sutton-Tyrrell et al found levels of aortic stiffness positively associated with cardiovascular mortality [97]. A study with (n=265) end-stage renal disease and diabetes subjects reported a positive association between aortic stiffening and cardiovascular as well as all-cause mortality [98]. In a Danish study, aortic PWV was a strong predictor of a composite of cardiovascular outcomes (cardiovascular mortality, coronary heart disease and stroke) beyond traditional cardiovascular risk factors [100].

Carotid-femoral pulse wave velocity (cfPWV) is a non-invasive, reproducible, an indirect measurement of central arterial stiffness [97,100]. Generally, once the pulse wave is generated at each contraction of the heart, it travels through out the arterial tree. The wave's speed depends on the functional and structural status of the arterial wall through which it is traveling. Incident wave travels away from heart while the reflective wave travels towards heart [101]. The slow speed of the wave (low PWV) suggests normal artery, while faster speed of the wave (high PWV) suggests stiffer arteries.

During diastole, a normal artery continuously ejects the blood through the arterial tree. On the other hand, stiff arteries can not accommodate the volume ejected by the left ventricle due to limited arterial elasticity, which in turn leads to increased pulse pressure and isolated systolic hypertension [102,103]. Arterial stiffness is an important determinant of systolic blood pressure, pulse pressure and precedes isolated systolic hypertension and is considered as an indicator of vascular aging [104].

2. 3   Adiposity and Atherosclerosis

Wealth of evidence suggests that obesity lays foundation for several chronic diseases including metabolic syndrome, diabetes, dyslipidemia, hypertension, coronary heart disease, stroke and some cancers [10,27,105]. Metabolic activity of adipocytes in pathogenesis of adverse health outcomes may vary depending on organ or tissue of localization rather than whole body fat [106]. Abdominal adiposity in particular, has been linked to increased cardiovascular morbidity and mortality[107].

Several studies have consistently linked obesity to significantly increased rates of mortality; however, there is a substantial variation in the actual number attributed to obesity across the studies. For the year 2000, Mokdad et al estimated 414,000 deaths due to obesity in US, whereas for the same period Flegal et al estimated only 112,000 deaths attributed to obesity [108,109]. Potential explanation for these variations may be because of different analytical approaches. Mokdad et al did not adjust for several variables such as hypertension, dyslipidemia, genetics etc while estimating the number of deaths due to obesity, which may have overestimated the number of deaths. Since the impact of obesity on health outcomes could be mediated through different biological mechanism, it may not be appropriate to attribute 400,000 deaths to obesity alone before adjusting such factors. Authors later acknowledged that the numbers were overestimated [110]. Similarly, apparent lower number of deaths in overweight category as compared to people in normal BMI range in Flegal et al's study may have been underestimated, because BMI range from 25 to less than 30 is an imprecise biomarker of body fat and physical activity [27,111].

Although, biological mechanism of obesity induced CVD is not fully understood yet, obesity is thought to be associated with CVDs through a number of biological mechanisms

including hypertension, dyslipidemia, insulin resistance, diabetes, endothelial dysfunction, inflammatory response, procoagulant, prothrombotic states etc [48]. However, some experts argue that obesity may have direct negative relation at least to some degree with CVD, in addition to mediation through established risk factors. Recently, two mechanisms, alteration in haemodynamics and ectopic fat deposition (in heart, blood vessel, kidney, liver and pancreas etc), are being considered as potential direct links between obesity and CVD [112-114]. Haemodynamic system in obese individuals, needs to work harder to meet a higher metabolic demand due to increased body size, there by increasing cardiac output and left ventricular stroke work [115,116] [116]. On the other hand, in animal models, significant increase in deposition of fat pads in heart, blood vessel and kidney have been observed [117,118]. It has been hypothesized that increased ectopic fat in heart may lead to increased ventricular stiffness and myocardial lipotoxicity. Higher fat deposition in blood vessel may release higher amount of growth factors acting on adjacent vascular smooth muscle cells and may secret pro-atherogenic cytokines, while accumulation of fat in kidney may also exert abnormal physical pressure within itself, promoting sodium retention, ultimately leading to cardiac dysfunction additively [112,119].

Recent findings suggest that obesity is also associated with a low-level inflammation resulting from chronic activation of the innate immune system and which can lead to insulin resistance, impaired glucose tolerance and diabetes [47,120-126]. Adipose tissue releases a variety of pro-inflammatory cytokines and chemokines, such as TNF-alpha, interleukin- 6 (IL-6), monocyte chemoattractant protein 1 and others [47]. When compared to adipose tissue of lean subjects, adipose tissue of obese subjects release higher amounts of TNF-alpha, IL-6, interleukin-8 (IL-8), transforming growth factor β1 (TGF-β1), C-reactive protein (CRP), soluble intercellular adhesion molecule (ICAM), monocyte chemotactic protein-1, and pro-coagulant

proteins such as plasminogen activator inhibitor type-1 and factor VII [127-129]. Visceral adipose tissue secretes higher amount of IL-6 and TNF-alpha. IL-6 stimulates lipolysis and hepatic triglyceride secretion ultimately leading to hyper triglyceridemia in vivo [130-132]. Another cytokine, IL-8 impacts directly on endothelial cells by attracting monocytes and promoting vascular smooth cell migration [133,134]. In the development of atherosclerosis, migration of monocytes is considered an integral part. Circulating monocytes adhere to the endothelial layer of vessel wall, migrate into vascular interstitium, and phagocytize oxidized LDL and form foam cells loaded with lipid [70]. Foam cells then start to accumulate within arterial wall forming early lesions called fatty streaks which then develop into advanced atherosclerotic plaques containing necrotic lipid cores surrounded by proteoglycan matrix and covered by a fibrous cap that thickens the intima. Also, pro-inflammatory factors minimize the beneficial effect of some cytokines and effect cellular metabolism. TNF-alpha decreases insulin sensitivity; suppresses genes involved in uptake and storage of NEFA and glucose, apoptosis as well as stimulates production of IL-1 and IL-6 [134]. TNF-alpha also exerts the inhibitory effect on adiponectin [135,136].

On the other hand, VAT has been found to release lower amount of adiponectin than subcutaneous fat in both lean and obese subjects [137,138]. Adiponectin has anti-diabetic, anti-atherosclerotic and anti-inflammatory characteristics [41,43]. Although, biological mechanism of beneficial properties of adiponectin is not fully understood yet, it has been believed to decrease circulating levels of free-fatty acids by increasing non-esterified free fatty acids (NEFA) oxidation in the skeletal muscle thus decreasing triglycerides in the skeletal muscle and reducing hepatic triglyceride deposition with resultant improved insulin sensitivity.

Obese individuals have substantially higher amount of fat stored in their adipose tissue, partly through the diminished inhibitory effects of insulin on lipolysis, to release free-fatty acid

in higher rates than non- obese individuals, even under normal physiological condition. Whether

higher amount of free-fatty acids flux in peripheral circulation and increased availability of free-

fatty acids to tissues play a role in impairing CVD functions is yet to be known [9]. For a module

depicting potential biological mechanism of obesity on the onset of CVD, see figure 2-6.
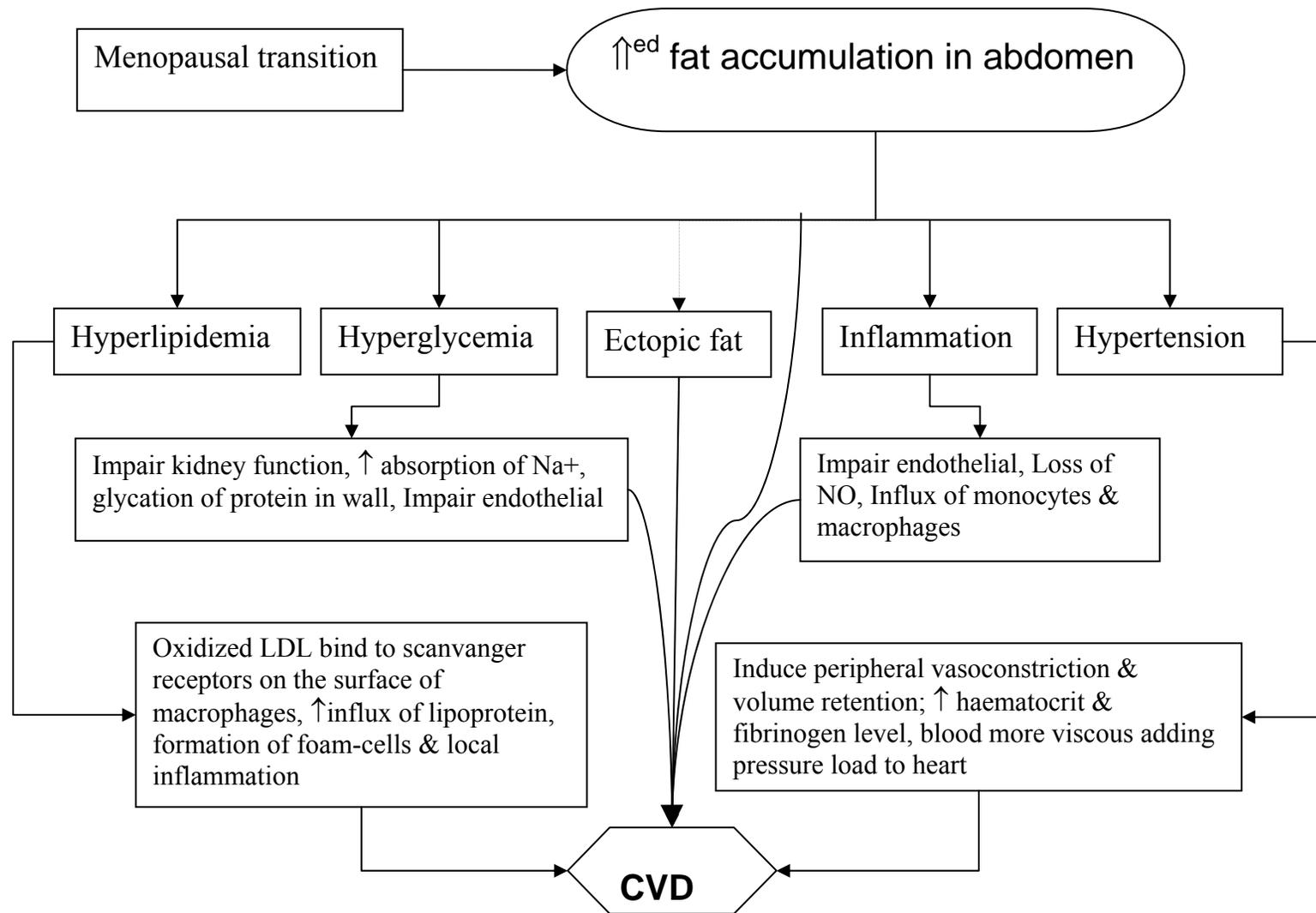
Figure 2-6 Potential biological link between abdominal adiposity and CVD

Differential roles of visceral and subcutaneous fat depots on CVD or subclinical CVD have been evaluated by many studies around the globe. Tyrrell et al found statistically significant association (p<0.0001) of abdominal fat with aortic stiffening measured by pulse-wave velocity in elderly American [139]. In a cross-sectional evaluation from the Amsterdam Growth and Health Longitudinal Study, at an early 36 years of age, abdominal and truncal subcutaneous fat were independently associated with arterial stiffness. However, the relationship between total adiposity and arterial stiffness was mediated by other established risk factors such as hyperlipidemia, hypertension etc [3]. Among Mexican healthy women, visceral adipose tissue significantly explained the variation of fasting glucose (28.4%), triglycerides (16.3%), LDL (15.8%), total cholesterol (12.4%) [4]. Similarly, another study has found VAT as an independent predictor of glucose metabolism among older postmenopausal healthy women [5]. Wildman et al found that BMI, body weight, waist and hip circumference, waist-to-hip ratio as strong correlates (<0.0001 for all) of pulse-wave velocity in younger (20-40 years of age) as well as older (41-70 years of age) Americans [46]. A Japanese cross-sectional study found BMI but not WHR is progressively and significantly associated with coronary stenosis among males (median age 59 years) and females (median age 67 years) who had coronary angiography. The study reported that the odds of presence of significant stenosis was highest (OR= 2.5, p for trend= 0.02) for the fourth quartile of BMI when compared to the first quartile of BMI [140].

2.4 Specific Aims

Although, the probability of dying from CVD at birth for any American is 47%, the risk of the disease is substantially increased for menopausal women and older adults. Atherosclerosis, which encompasses two aspects of vascular changes; thickening of arteries due to plaque accumulation (atheroma) and hardening of arteries (sclerosis) is a major determinant of CVD events. Being non-invasive, reliable and cost-effective, carotid IMT, AD and cfPWV are considered very important in assessing the status of atherosclerosis in subclinical stage.

Obesity, particularly abdominal adiposity has been suggested to be a major contributor of atherosclerosis and also has been shown to have a strong positive association with CVD events across the globe. In contrast, peripheral adiposity is indicated to have a negative correlation with atherosclerosis. Although anthropometric measures are most commonly used markers of adiposity in clinical as well as research settings, because of its cost and ease of use, they have limited ability to differentiate the contribution of fat and lean mass on disease outcomes. Hence, newer technologies DXA and CT that are able to differentiate fat and lean mass have been suggested as more precise and accurate in measuring total and regional distribution of fat and lean mass and to estimate their contribution to disease outcomes. However, whether the newer technologies are superior in explaining variability in S-CVD over anthropometric markers, particularly BMI, WC and WHR is not clear yet. Hence, the specific aims of this dissertation are as follows;

I. To investigate a cross sectional relationship of total and regional fat distribution with carotid IMT and AD among women passing trough menopausal transition,

II. To compare the variances explained by different measures of total and regional adiposity on carotid IMT and AD in the same cohort mentioned above and,

III. To compare the variances explained by different measures of total and regional adiposity on cfPWV in the above mentioned cohort.

3.0 Impact of total and regional fat distribution on carotid intima media thickness and on carotid adventitial diameter of women in menopausal transition- a cross-sectional evaluation from SWAN- Heart study

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(Manuscript in preparation)

3.1 Abstract

Objective:

To examine the associations of total and regional body fat distribution with the carotid intima media thickness (IMT) and adventitial diameter (AD) among women in the menopausal transition.

Method:

We measured total and regional fat distribution by dual energy x-ray absorptiometry (DXA) and, IMT and AD by B-mode ultrasound in 197 healthy Caucasian (70%) and African American (30%) women (mean age 50.1 years) participating in an ancillary study to the Study of Women's Health Across the Nation (SWAN).

Results:

After adjusting for age, race, menopausal status, insulin, systolic blood pressure (SBP), triglycerides, height, high-density lipoprotein (HDL) and smoking status, proportion of higher total fat relative to weight was associated with larger AD (beta for each percent increase in total fat 0.011, p= 0.03). Proportion of trunk fat relative to total fat was also positively associated with AD (beta for each percent increase in trunk fat= 0.014, p= 0.03). In contrast, proportion of leg fat relative to total fat was associated with smaller AD, (beta for each percent increase in leg fat= -0.013, p= 0.03). Women in late peri or post menopausal status had significantly larger AD compared to women in pre or early peri menopausal status after adjusting for trunk fat (p= 0.03). Significant associations of total and regional fat distribution with IMT became non-significant after adjusting for SBP.

Conclusion:

Adiposity is directly associated with AD and may be indirectly associated with IMT through SBP. Also, the accumulation of fat in different anatomical regions may have a differential role in subclinical atherosclerosis; greater accumulation of fat in the trunk appears to have more negative impact on vascular health than fat in the legs.

3.2 Introduction

Anthropometric markers of obesity are positively associated with cardiovascular disease (CVD) and with traditional risk factors of CVD including; hypertension (HTN), hyperlipidemia, type II diabetes and atherosclerosis [10,30,45,46,109,140-142]. Recent studies using more precise markers of adiposity, distinguishing fat from fat-free mass, indicate opposing roles of abdominal and peripheral adiposity on atherosclerosis or its risk factors [6,8,49,50,105,143]. For example, higher abdominal adiposity has been found to be more strongly associated with increased clinical or subclinical CVD than peripheral (leg and arm) or overall adiposity [33,35,139,144-146]. A number of studies have suggested that mesenteric, pre-peritoneal and subcutaneous fat are positively associated with carotid intima media thickness (IMT), a widely accepted marker of atherosclerosis[147,148], while others have found lower risks of atherosclerosis with increased peripheral fat mass, particularly fat mass in lower extremities [7,8,49,50].

Although anthropometric measures are inexpensive and easy to obtain, they provide only approximate estimates of body composition and have limited ability to measure fat mass distribution by anatomical region. Thus they may have limited significance in terms of disease outcomes. Dual energy x-ray absorptiometry (DXA) is a more sensitive technology that has the ability to differentiate adipose tissues from lean tissues and bones. Because of its ability to measure body fat with low radiation and its better applicability in large population studies than other instruments (computed tomography, magnetic resonance imaging), DXA is now considered the gold standard for measuring total and regional distribution of adipose tissue [26]. DXA differentiates fat mass in body compartments including legs, arms, head and trunk. Exploring relationships of fat in the trunk, legs and arms measured by DXA, with subclinical CVD may help to elucidate the impact of fat distribution on subclinical CVD.

The prevalence of overweight and obesity among females reaches its peak between the ages of 45 and 50 years, the age range when most women experience the menopausal transition and decreases after the age of 65 years [11]. Recent findings suggest that postmenopausal women tend to have a higher percentage of torso fat than pre-menopausal women, probably due to a shift in peripheral fat or loss of lean mass [51,52,56-59,62,68,149-152]. Similarly, the risk of CVD increases substantially among women ten years after menopause. Decline in many female hormones at menopause may be responsible, at least partly, for increased risk of numerous negative health outcomes including android fat accumulation and CVD[153-155]. These changes in women's life may not just be the function of aging, as perceived by general population, but indicate some important yet unknown role of physiological phenomenon. Little is known about regional fat distribution and its role in subclinical CVD among women during the menopausal transition. Most of the available information regarding the relationship of total and regional fat distribution and subclinical CVD are based on pre- versus post-menopausal status. It is important to evaluate fat partition and its impact on subclinical CVD during the menopausal transition.

Hence, the objective of this paper is to examine associations of body composition; total and regional body fat distribution measured by DXA with subclinical measures of atherosclerosis measured by IMT and adventitial diameter (AD) in healthy women passing through the menopausal transition.

3.3 Design and Methods

3.3.1 Study population

Details of the study design and population have been published previously [156]. In brief, the study of Women's Health Across the Nation (SWAN) is a multi-center, multi-ethnic, longitudinal epidemiological study studying both the physical and psychosocial changes women experience during the menopausal transition. Women were recruited (n= 3,302) at seven study sites. Each study site recruited Caucasian and one other ethnic group; African American (Boston, MA; Chicago, IL; Detroit, MI; Pittsburgh, PA), Hispanic (Newark, NJ), Japanese (Los Angeles, CA) and Chinese (Oakland, CA). Main eligibility criteria for enrollment were: a.) an intact uterus and at least one ovary, b.) at least one menstrual period in previous 3-months, c.) no use of reproductive hormones in previous 3-months and d.) 42-52 years of age.

SWAN- Heart is an ancillary study of SWAN, designed to examine risk factors for sub-clinical CVD in African American (AA) and Caucasian American (CA) women in menopausal transition. Six hundred and eight SWAN participants were enrolled in SWAN-Heart at the Pittsburgh and Chicago field centers. SWAN participants were eligible for SWAN-Heart unless they: a.) were taking medication for diabetes, hypertension or heart arrhythmias, b.) had a history of clinical atherosclerosis (MI, angina, intermittent claudication, cerebral ischemia or revascularization), c.) had had a hysterectomy and/or bilateral oophorectomy or d.) were taking any female hormones. A single baseline SWAN- Heart study was conducted for each participant in conjunction with her core SWAN interview at annual follow-up visits 4, 5, 6 or 7.

Five SWAN sites including Pittsburgh but not Chicago performed annual DXA scans. Therefore, the present study is based on data obtained from Pittsburgh only. Figure 3-1 provides details related to which SWAN-Heart participants were included in the present analysis. The

Institutional Review Board at the University of Pittsburgh approved the SWAN and SWAN-Heart studies.

3.3.2 Measurements

3.3.2.1 Body fat measurements

DXA methodology determines bone, fat and lean masses based on energy attenuation differences. DXA scans were performed by a trained and certified technician, following standard protocol on a Hologic QDR 2000 machine in fan-shaped-array mode. Participants were advised not to take any medication or supplements containing calcium for at least 24 hours before the scan. Subjects reporting the use of x-ray using contrast or any nuclear medicine for one week prior to the scan date and those found positive on pregnancy test were not scanned. Participants were asked to remove all removable metal objects from the body. The coefficient of variation of measurement was 2.1%.

3.3.2.2 Subclinical atherosclerosis measurements

For the present study, a duplex scanner, Toshiba SSA-270A was used to measure the IMT and AD of carotid artery. AD was measured in the CCA while IMT was measured at multiple sites of carotid artery. Images for IMT were taken from near and far walls of the distal common carotid artery, CC bifurcation and the first centimeter of the internal carotid artery. Digitized images were quantified using Artery Measurement System (AMS) software, developed in Sweden. The AD of the common carotid was measured directly as the distance from the adventitial-medial interface on the near wall to the medial-adventitial interface on the far wall at end-diastole. Reproducibility of measures was evaluated by replicating readings on 20 scans from these women. The intra-class correlation was 0.98 for IMT and 0.99 for AD.

3.3.2.3 Menopausal status

Women with a menstrual period in the past three months with no change in regularity in the past 12 months were categorized as pre-menopausal, while women with some change in regularity over the previous 12 months were classified as early perimenopausal. Late perimenopausal status was assigned to women with no menstrual period within the past 3-months, but some menstrual bleeding within the past 12 months and post menopausal status was assigned to women who did not have menstrual period within the past 12 months. Women reporting the use of birth control pills, estrogen, estrogen injection/ patch, combination estrogen/ progestin, or progestin pills in the past year were considered hormone therapy users. Women with hysterectomy with or with out removal of one or both ovaries were grouped in surgical menopause category. Hormone therapy users and women who had surgical menopause were excluded from current analysis. Menopausal status was dichotomized by combining premenopausal and early perimenopausal in one group and late peri- and postmenopausal into another group, because of the small number of women in premenopausal (n= 20) and late perimenopausal (n= 22) categories.

3.3.2.4 Additional measures

Socio-demographic variables including race, age, education level, smoking status and physical activities were collected at the initial SWAN study visit. Smoking status was categorized as current smoker, past smoker or never smoker. Participants were asked to wear light clothing and take off shoes while measuring height, weight and WC. BMI was calculated as the ratio of weight in kilograms to height in meters squared. Two blood pressure readings were taken in right arm with the participant seated, following at least 5-minutes of rest. The average of

these two measurements was used in the analysis. Weekly calibration of portable scales and monthly calibration of study clinic based stationary scales were performed.

3.3.3 Statistical methods

Analyses were done using SAS version 9.1, SAS Institute, Inc., North Carolina, USA. Normality assumptions for continuous and cell count for categorical data were checked. Statistical signifcance was based on a p-value of 0.05.

For current analysis, we used the DXA whole body scan, performed in conjunction with the core SWAN visit corresponding to the participant's baseline SWAN- Heart exam. Descriptive statisitics were described in terms of mean ± standard deviation for continuous variables and proportions for categorical variables. Categorical variables were compared using Chi-square and continuous variables were compared using Analysis of Variance (ANOVA) or Kruskal-Wallis test, across tertiles of trunk fat mass. For regression analysis, IMT was normalized using log transformation.

Unadjusted correlations of IMT and AD with body composition variables were calculated. Associations of independent (body compostion) and dependent (IMT and AD) variables were assessed with univariate linear regression. Covariates that were signficantly associated (p<0.05) with dependent variables in univariate regression models were grouped by similarity of the variables and further tested in regression model to identify the variable with the most significant association using stepwise selection. For example, diastolic blood pressure (DBP), systolic blood pressure (SBP) and heart rate were grouped in one model, while total cholesterol, low density lippoprotein (LDL) and triglycerides were grouped in another model. Since high density lippoprotein (HDL) is suggested to play protective role on subclinical CVD,

opposite to other lipid markers, HDL was not included in the lipid group. Similarly, glucose and insulin were included in another model. The base model was developed retaining variables that were most significant in group models; SBP, triglycerides and insulin, and adding age, height, menopausal status, race, HDL and smoking status.

Total leg and arm fat masses were obtained by adding left and right leg fat mass, and left and right arm fat mass respectively. Total body fat was calculated by adding trunk, total arm and total leg fats, excluding fat in the head. Instead of absolute fat mass, proportion of total and regional fat distributions were used in the regression models. Proportion of fat in a specific anatomical region was calculated as percentage of total fat mass (e.g., proportion of trunk fat= trunk fat mass x 100/ total fat mass), while proportion of total fat was calculcated as percentage of total body weight; total fat mass x 100/ weight.

In final multivariate models, log IMT and AD were modeled separately using proportions of trunk, leg, arm, or total fat as independent variable, adjusting for the effect of age, height, SBP, smoking status, menopausal status, HDL, insulin and race. Regional proportions of fat were adjusted for corresponding proportions of lean mass in addition to above mentioned variables. Since anthropometric measures were strongly correlated with DXA adiposity markers; weight, BMI and WC were not included in the multivariate model.

Changes in R^2 were compared to identify the best predictor of outcome variables among markers of regional adiposity by adding them to the base model. Absolute regional fat masses rather than the proportions of fat were used to minimize the colinearity effect, because the variance inflation ratio associated with fat masses were substantially lower than those associated with proportions of fat (22.0 vs 3.5).

3.4 Results

Out of 259 women with written consent to participate in the ancillary study, 21 did not complete carotid scans and 10 women participated in carotid scan did not participate in DXA scans. An additional 31 women (AA= 6 and CA= 25) were excluded because of surgical menopause (n=11), hormone replacement therapy use (n= 19) or missing menopausal status (n= 1).

Basic characteristics of the study population by tertiles of trunk fat mass are given in Table 3-1. A total of 197 women (30% AA and 70% CA) had complete data for the current analysis. Mean age of the participants was 50.1 years. Sixty percent of participants were in pre- or early perimenopausals status. Means of CCA AD and IMT were 6.6 mm and 0.70 mm, respectively.

The odds of being in upper tertile of trunk fat was significantly higher for women with larger AD, thicker IMT, higher total, arm and leg fat masses. Similarly, heavier women and women with higher BMI, WC, total cholesterol, LDL, triglycerides, DBP, SBP, insulin, glucose and C-reactive protein had significantly higher odds of being in the upper tertile of trunk fat mass (Table 3-1). A statistically significant odds of being in upper tertile of trunk fat mass was observed among women who had lower HDL level. Distribution of smoking status was significantly different by tertile of trunk fat (figure 3-2).

Characteristics of women excluded from current analysis were not significantly different from that of women included in the analysis except mean age. Excluded women were found to be older than women included in analysis (p= 0.03).

3.4.1 IMT

Univariate correlation analysis:

In an unadjusted correlation analysis, log of carotid IMT was significantly and positivley correlated with proportions of; arm fat, trunk fat and total fat. In contrast, proporiton of leg fat was negatively correlated with log of IMT. Similarly, log IMT was positively correlated with proportion of leg lean while negatively correlated with proportions of trunk and total lean masses. Weight, BMI and WC were also positively correlated with log IMT (Table 3-2).

Multivariate regression analysis:

None of the markers of total or regional adiposity were found to be significantly associated with log IMT after adjustment for age, height, SBP, insulin, triglyceride, HDL, menopausal status, race and smoking status. However, SBP and insulin levels were positively and significantly associated with log IMT in all models (Table 3-3). The association between trunk fat and log IMT did not remain statistically significant after adjusting for SBP.

3.4.2 CCA AD

Univariate correlation analysis:

Proportions of trunk fat and total fat were positively and significantly correlated, whereas proportion of leg fat was negatively correlated with AD in unadjusted correlation analyses. Other variables that had positive correlation with AD were weight, BMI, WC. Proportions of trunk and total lean were negatively but proportion of leg lean was positively correlated with AD. Age and AD tended to be positively correlated , however the relationship was marginally significant (p= 0.07).

Multivariate regression analysis:

Proportion of total fat adjusted for above mentioned covarites were positively associated with increased AD, p= 0.03. After adjustment for proportion of trunk lean in addition to the above mentioned covariates, proportion of trunk fat was also positively associated with enlarged AD, p= 0.03. In contrast, proportion of leg fat (p= 0.03) was negatively associated with AD, after adjusting for covariates and proportion of leg lean.

Also, in all regression models with proportions of total or regional fat, women who were classified as current smokers appeared to have significantly (p-value from 0.01 to 0.02) larger AD when compared to women who were never smokers. AD of the women in later menopausal stage was found significantly larger, in all models than that of women in earlier menopausal stage, p-value from 0.03 to 0.04 (Table 3-3).

To identify the best predictor of AD among regional markers of fat distribution, we compared the change in R^2 while adding regional fat masses to the base model. Trunk fat mass (R^2= 0.286) explained the most variance in AD, while leg or arm fat mass did not provide more information in the model than trunk fat mass (Table 3-4).

3.5   Discussion

In this study, we investigated the association of total body fat and regional fat on average carotid IMT and the diameter of the CCA adventitia among healthy women undergoing menopausal transition. We found a higher proportion of total fat relative to body weight and a higher proportion of trunk fat relative to total fat were each associated with enlarged adventitial diameter of the common carotid artery. In contrast, a higher proportion of leg fat relative to total body fat was associated with smaller AD. In our analysis, trunk fat mass explained greater variance in CCA AD than arm or leg total fats. However, none of the markers of total or regional adiposity were independently associated with IMT after adjusting for age, race, menopausal status, height, SBP, insulin level, HDL, triglyceride and smoking status.

The majority of prior studies evaluating the relationship of body size with vascular health have used anthropometric measurements such as BMI [142,155,157], WC [158], WHR [159], abdominal diameters [160]. Our findings are consistant with their results. Wildman et al found WC and BMI positively associated with CCA AD in US young adult population[46], while Jensen-Urstad et al reported BMI associated with CCA diameter among Swedish females but not males [87]. Both of these studies had fewer women than our study. Participants were younger (age range 20-40 years) in the former study and older (mean age 55 years) in the later study compared to our study. More importantly, we examined similar relationships but with more precise measure of adiposity using DXA methodology among women in menopausal transition. Another large study (n= 1,014) from Germany found positive association of lumen diameter with BMI, WC, fat mass (using bio-inpedance methodology) and fat-free mass [158]. In present analysis among US women only, we used AD diameter (lumen plus intima-media thickness) rather than just lumen diameter. In Healthy Women study, Patel et al found weight as an independent major risk factor of

increase in aortic diameter among post-menopausal women [161]. They used abdominal and iliac adventitial diameters, whereas we used CCA AD of women in menopausal transition.

To our knowledge, present study is the first to report an significant association of total, trunk and leg fat depots with AD among women in menopausal transition.

Relationship of adiposity and subclinical atherosclerosis has been found consistently. In particular, abdominal fat accumulation is suggested to increase the risk for CVD mortality and morbidity more than any other regional fat depots [26,33,49,144,162]. Consistent with this concept, current study shows a positive and statistically significant relationship between abdominal adiposity and enlargement of CCA AD. Recent studies indicate that enlargement of AD may precede IMT thickening and thus it may be a very early stage of atherosclerosis [93,94,96]. Also in our study, proportion of trunk fat explained more variance in AD than proportions of leg or arm fats. This may be because of the capacity of visceral adipose tissue to deliver higher amount of free fatty acids by releasing them directly into the circulation system [26,105,163]. Also, adiposity is associated with release of cytokines which may in turn play important role in the progression of atherosclerosis. Abdominal adiposity has been suggested to be inversely correlated with adiponectin and positively correlated with inflammatory markers including interleukin- 6 (IL-6), tumor-necrotizing factor α (TNF- α) [26,38,105]. It has been suggested that IL-6 and TNF-α contribute to increased inflammation, while lower adiponectin level may play role in increased insulin resistance. Both of these biological proccesses are believed to be independently involved in the progression of atherosclerosis [26,38]. In addition, a number of other biological mechanisms including alteration in renin-angiotensin system [164], or increased leptin levels [165] have been observed with increasing abdominal adiposity, which may increase fluid volume by retaining sodium, indirectly leading to enlarged AD [166,167 168].

On the other hand, prior studies have suggested an inverse relationship of peripheral adiposity, particularly fat accumulation in the lower extremities, and atherosclerosis [8,50]. Consistent with this hypothesis, our analysis also revealed that proportion of leg fat relative to total fat was negatively and significantly associated with AD. A recent study found negative correlation between fat in lower extremities and, glucose and lipid levels (risk factors of atherosclerosis) in elderly population [6]. A small study with 95 postmenopausal women reported a negative association of leg fat with fasting insulin and triglycerides, both risk factors for subclinical CVD, even after controlling for trunk fat (accessed by DXA)[7]. Similarly, other studies also found lower levels of atherogenicity with increased peripheral fat mass and leg fat percentage [8,49,50]. Although, the mechanism involved in the observed negative association of leg fat and cardiovascular risk profile is largely unknown, a few hypotheses have been suggested. Adipocytes in the leg, particularly in the femoral-gluteal region, are relatively sensitive to anti-lipolytic activity while being less sensitive to pro-lipolytic activity, limiting the release of free fatty acids [169]. This increased uptake of free fatty acids in femoral-gluteal region may limit the ectopic fat deposition in the blood vessel, thus limiting the release of vasoactive substances including inflammatory markers, ultimately helping AD to maintain its structure.

Measures of total and regional proportion of fat were not associated to IMT after adjusting for age, race, menopausal status, height, SBP, insulin, HDL, smoking status and triglyceride. Previous studies assessing the association of total body fat or regional fat distribution on IMT are inconsistent. Studies that reported an independent positive association of total or regional adiposity with IMT have mostly used anthropometric measurements such as weight [161], BMI [155], WC [144]. In contrast, other studies using more precise markers of regional fat distribution such as sagittal or transverse diameters [160], visceral fat area [148], fat mass [3] did not

find significant association after adjusting for potential confounders and/or covariates similar to our's. A cross-sectional study which is very close to the present study in terms of outcome and predictor measurements, did not find significant realtionship between CCA IMT and fat mass after adjusting for lean mass [3]. In our study, the association of total and regional proportions of fat with IMT lost statistical significance when we added SBP in the model. This may indicate that the impact of adiposity on atherosclerosis in this population might be mediated via other biological mechanism such as hypertension.

The observed positive association of markers of regional fat distribution with AD and lack of such association with IMT may be best explained by the theory that AD enlargement precedes IMT thickening[93]. Recent work in animal models suggest that enlargement of AD may precede IMT thickening, especially in case of vascular injury in coronary arteries[94,170]. Alternatively, it is possible that the mean value of IMT in our study is below the cut point for 'high risk'. Although a widely-accepted exact cut-off value of IMT still needs to be defined, experience from prior epidemiological and observational studies indicate that CCA IMT of 1.0 mm or more is considered high risk group for future CVD events in general population[73,75,76,83]. Since our study population is in 'very early' stage of atherosclerosis as indicated by mean CCA IMT of 0.70 mm, a signifcant association of adiposity with IMT may not be apparent at this point.

Relatively small sample size, smaller proporitons of women in premenopausal and late peri-menopausal status were the main limitations of the present study. Since the present study is cross-sectional by design, the results can not be inferred as causal.

3.6   Conclusion

Current study suggests that accumulation of fat in different anatomical regions possess direct or indirect differential roles in subclinical atherosclerosis among women in menopausal transition. Abdominal fat accumulation may be related to increased IMT through hypertension, while it may be directly associated to the enlargement of AD. Overall, fat accumulation has negative impact on subclinical atherosclerosis, however retaining higher proportion of fat in the lower extremities relative to the total body fat may have favorable impact.
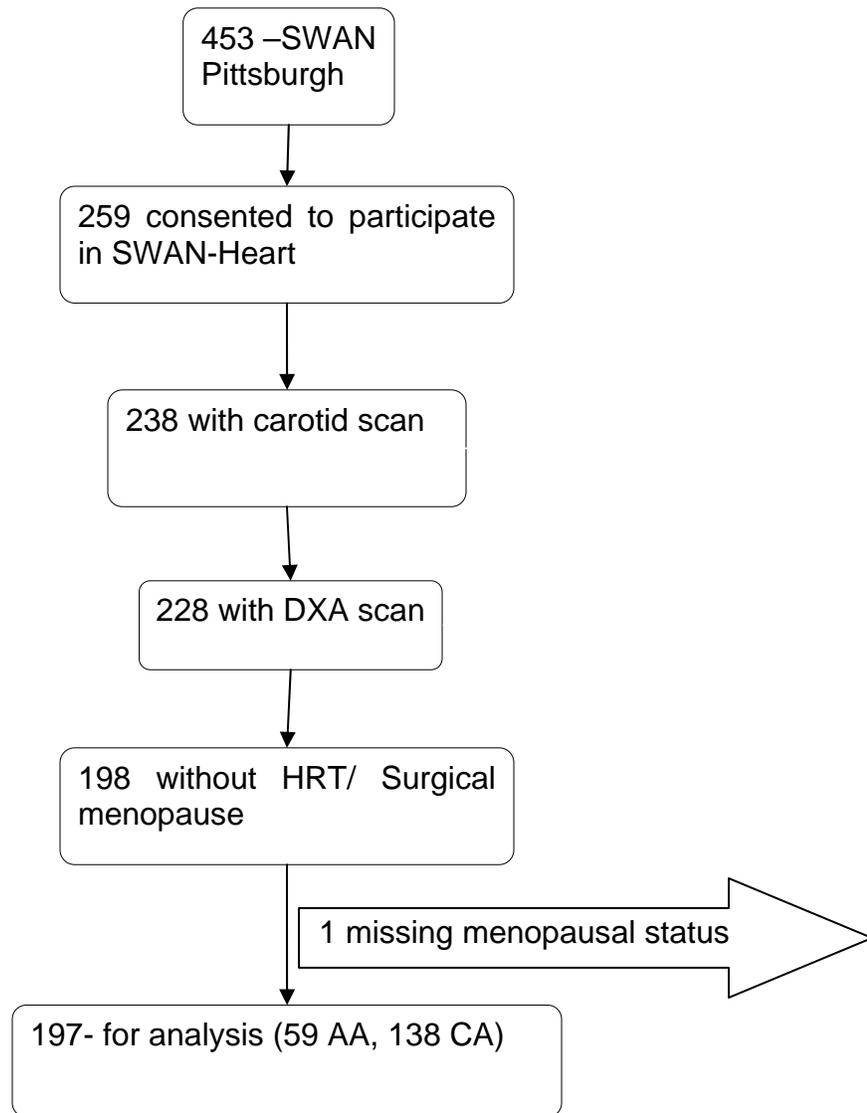
Figure 3-1. Flow chart of women included in the analysis.

Table 3-1. Participant characteristics by trunk fat[#]

Characteristics	Combined N= 197	1st tertile (2.2 – 10.4) N= 65	2nd tertile (10.4 – 16.4) N= 66	3rd tertile (17.1 – 37.2) N= 66	P-value
		Tertile of trunk fat mass (kg)			
IMT (mm)	0.70 ± 0.09	0.67 ± 0.07	0.69 ± 0.08	0.72 ± 0.11	**0.021**
AD (mm)	6.6 ± 0.6	6.4 ± 0.5	6.7 ± 0.6	6.8 ± 0.6	**<0.001**
Arm fat total (kg)	4.5 ± 1.9	2.8 ± 0.7	4.1 ± 0.8	6.5 ± 1.5	**<0.001**
Leg fat total (kg)	12.8 ± 4.2	9.4 ± 2.5	12.5 ± 2.8	16.1 ± 3.9	**<0.001**
Total fat (kg)	31.8 ± 12.4	19.6 ± 4.5	29.8 ± 4.2	45.9 ± 8.6	**<0.001**
African American (%)	30.0	16.9	43.9	28.8	0.169
Age (years)	50.1 ± 2.6	50.1 ± 2.6	49.8 ± 2.6	50.3 ± 2.5	0.630
Weight (kg)	76.7 ± 16.9	61.1 ± 7.1	74.1 ± 6.9	94.6 ± 13.9	**<0.001**
BMI (kg/m^2)	28.9 ± 6.1	23.2 ± 2.1	27.8 ± 2.6	35.6 ± 4.8	**<0.001**
Waist (cm)	88.6 ± 14.5	75.0 ± 5.7	86.7 ± 7.1	104.3 ± 10.8	**<0.001**
Cholesterol (mg/dl)	197.1 ± 34.1	186.0 ± 28.4	195.6 ± 38.9	209.6 ± 30.4	**<0.001**
LDL (mg/dl)	120.5 ± 30.0	110.9 ± 25.4	120.1 ± 33.7	131.4 ± 27.1	**<0.001**
HDL (mg/dl)	54.6 ± 12.6	58.5 ± 13.9	56.4 ± 12.1	48.9 ± 9.8	**<0.001**
Triglycerides (mg/dl)	105.5 ± 60.1	82.3 ± 28.8	95.4 ± 49.7	141.0 ± 77.3	**<0.001**
Glucose (mg/dl)	91.4 ± 14.6	87.4 ± 8.0	90.3 ± 8.7	96.7 ± 21.7	**<0.001**
Insulin (uIU/ml)	9.8 ± 6.3	6.9 ± 3.7	9.2 ± 4.9	13.5 ± 7.9	**<0.001**
CRP (mg/ l)	3.4 ± 5.1	2.0 ± 3.9	2.6 ± 2.8	5.6 ± 7.6	**<0.001**
DBP (mmHg)	72.5 ± 8.6	67.2 ± 7.5	75.0 ± 8.7	75.3 ± 7.3	**<0.001**
SBP (mmHg)	114.2 ± 15.2	105.0 ± 10.5	117.3 ± 16.1	120.0 ± 14.0	**<0.001**
Menopausal status (%)					
Later	40.1	33.9	48.5	37.9	0.652
Education (%)					
<High school	1.0	0.0	1.5	1.5	
High school	21.8	21.5	24.2	19.7	
>High school	32.5	24.6	39.4	33.3	0.294
College	18.9	20.0	13.6	22.7	
Post graduate	25.9	33.9	21.2	22.7	

[#]- Values are expressed as proportions (categorical) and mean ± standard deviation (continuous).
 Later menopausal status = late peri- + post and earlier menopausal status = pre + early peri-
 P-values are based on ordinal logistic regression.
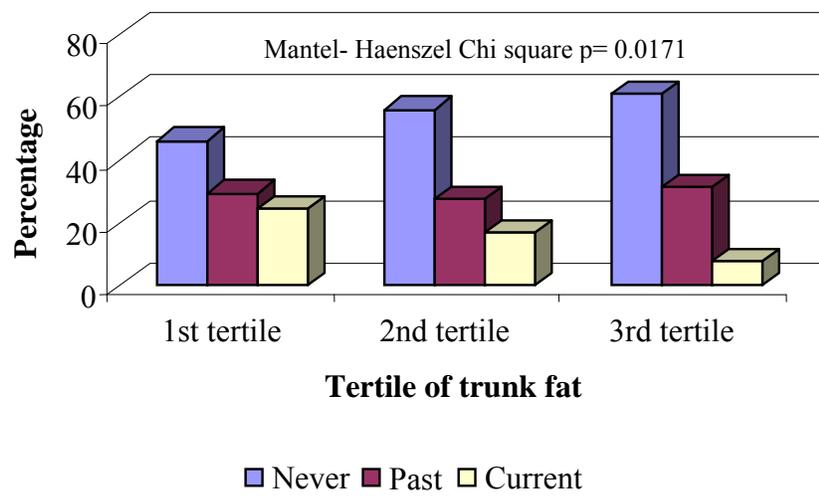
Figure 3-2. Smoking status by tertile of trunk fat

Table 3-2: Correlation of IMT and AD with body composition variables- (Spearman's)

| Predictors | Subclinical CVD markers (n= 197) | | | |
| | Average IMT | | AD | |
	Corr coeff	p-value	Corr coeff	p-value
Age (yrs)	0.13	0.062	0.13	0.067
Weight (kg)	0.28	**<0.001**	0.41	**<0.001**
BMI (kg/m^2)	0.31	**<0.001**	0.37	**<0.001**
WC (cm)	0.36	**<0.001**	0.39	**<0.001**
Arm fat (%)	0.16	**0.026**	0.11	0.142
Leg fat (%)	-0.21	**0.003**	-0.32	**<0.001**
Trunk fat (%)	0.19	**0.009**	0.32	**<0.001**
Total fat (%)	0.20	**0.005**	0.29	**<0.001**
Arm lean (%)	0.13	**0.061**	0.19	**0.007**
Leg lean (%)	0.18	**0.011**	0.25	**<0.001**
Trunk lean (%)	-0.22	**0.002**	-0.30	**<0.001**
Total lean (%)	-0.19	**0.007**	-0.29	**<0.001**

*Corr coeff- correlation coefficient.

Table 3-3: Association of IMT and AD with total and regional fat proportions

Variables	Log IMT	AD
Base Model	β (p-value) $R^2= 0.2248$	β (p-value) $R^2= 0.2251$
Age (yrs)	0.002 (0.58)	0.001 (0.94)
African American	-0.011 (0.60)	0.133 (0.17)
Later menopausal status	0.021 (0.32)	**0.201 (0.04)**
Height (cm)	0.003 (0.65)	0.017 (0.575)
SBP (per 10 mmHg)	**0.024 (<0.001)**	**0.085 (0.003)**
Insulin (uIU/ml)	**0.005 (0.005)**	0.012 (0.13)
HDL (mg/ dl)	-0.014 (0.08)	-0.026 (0.47)
Current smokers	0.007 (0.78)	**0.258 (0.02)**
Triglyceride	-0.002 (0.33)	0.0047 (0.57)
Model I[*]*-*	$R^2= 0.2248$	$R^2= 0.2450$
Later menopausal status	0.020 (0.32)	**0.203 (0.03)**
Total fat (%)	0.00007 (0.95)	**0.011 (0.03)**
Model II[#]*-*	$R^2= 0.2259$	$R^2= 0.2622$
Later menopausal status	0.021 (0.31)	**0.199 (0.03)**
Trunk fat (%)	-0.0007 (0.61)	**0.014 (0.03)**
Model III[#]*-*	$R^2= 0.2284$	$R^2= 0.2294$
Later menopausal status	0.020 (0.35)	**0.193 (0.04)**
Arm fat (%)	0.004 (0.39)	0.003 (0.89)
Model IV[#]*-*	$R^2= 0.2253$	$R^2= 0.2551$
Later menopausal status	0.020 (0.33)	**0.201 (0.03)**
Leg fat (%)	0.0003 (0.84)	**-0.013 (0.03)**

*- adjusted for age, race, menopausal status, height, SBP, insulin, HDL, triglyceride and smoking status.

#- adjusted for respective proportions of lean in addition to variables mentioned in a.

Later menopausal status = late peri- + post- and earlier menopausal status = pre- + early peri-.

Table 3-4: Change in R^2 on AD by adding regional fat mass*

Variance explained	Base model	Trunk fat	Trunk + leg fat	Trunk + arm fat	Trunk + arm + leg fat
R^2	0.224	0.286	0.289	0.287	0.290

*- Absolute fat masses without adjusting lean masses.

4.0 Waist circumference, proportion of trunk fat and area of visceral adipose tissue explain similar variability in subclinical atherosclerosis

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(Manuscript in preparation)

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4.1   Abstract

Objective

To compare the variance explained by three methods of adiposity measurement with carotid IMT and adventitial diameter among women transitioning through menopause.

Methods

We investigated 209 healthy Caucasian (69.44%) and African American (30.6%) women (mean age 50.3 ± 2.6 years) participating in the Study of Women's Health Across the Nation-Heart (SWAN-Heart) study. Carotid intima media thickness (IMT) and adventitial diameter (AD) were measured by B-mode ultrasound. The variability explained by weight, body mass index (BMI), waist circumference (WC) and, proportions of total, trunk, leg and arm fat masses (measured by dual-energy x-ray absorptiometry (DXA)), and areas of visceral (VAT), subcutaneous (SAT) and whole (WAT) adipose tissues (measured by computed tomography (CT)) on IMT and AD were compared using multivariate linear regression.

Results

Multivariate regression model with WC explained slightly more variance in IMT ($R^2 = 0.252$) than the models with VAT, SAT, WAT, proportion of trunk, total, arm and leg fat and, BMI, weight and WHR after controlling for age, race, height, menopausal status, LDL, HDL, insulin and SBP. Among all measures of adiposity, WC was the only variable positively and significantly associated with IMT. Regression models with weight ($R^2 = 0.275$), BMI ($R^2 = 0.271$) and WC ($R^2 = 0.270$) explained comparable variance in AD, while models with VAT ($R^2 = 0.258$) and, proportions of leg ($R^2 = 0.253$) and trunk fat ($R^2 = 0.251$) both relative to total fat, and proportion of total fat ($R^2 = 0.248$) relative to weight explained slightly lower variance in AD

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than by anthropometric measures. Weight (p<0.001), BMI (p=0.001), WC (p<0.001) and, proportion of total (p=0.02) and trunk (p=0.03) fat and, VAT (p=0.007) and area of whole adipose tissue (p=0.04) were positively associated with AD. In contrast, proportion of leg fat (p=0.03) relative to total fat was negatively associated with AD.

Conclusion

We did not find evidence to support CT or DXA measured measures of adiposity are better than anthropometric measures in explaining variances in carotid IMT and AD. WC – which is simple, inexpensive, readily available and easy to use - may be better or at least as good as VAT or proportion of trunk fat relative to total fat in explaining variability in IMT and AD. Abdominal accruement of fat may have more negative impact on S-CVD than added fat in the legs.

4.2   Background

There is a general consensus in the scientific community that increased adiposity is associated with a number of metabolic as well as cardiovascular diseases (CVD)[1-6]. Reported methodologies in available literature to measure adiposity include anthropometric measurements, sagittal diameters, dual energy x-ray absorptiometry (DXA), bio-impedance, air displacement plethysmography, computed tomography (CT), magnetic resonance imaging. Adiposity, mainly assessed by anthropometric measures is positively associated with CVD, subclinical cardiovascular disease (S-CVD) as measured by intima-media thickness (IMT) or adventitial diameter (AD), and their risk factors[7-16]. Although anthropometric measures are the most commonly used measures of adiposity in clinical as well as research settings due to low cost and ease of use, they are unable to differentiate between fat and lean mass. Hence, newer technologies DXA and CT that are able to differentiate fat and lean mass have been suggested as better ways to estimate the variable effect of fat and lean mass on disease outcomes [17,18]. However, the superior ability of DXA and CT to explain variability in S-CVD compared to anthropometric measures particularly body mass index (BMI), waist circumference (WC) and waist to hip ratio (WHR) is not clearly established. Although prior studies indicate fat mass is positively and fat free mass is negatively associated with CVD[2,3,19-22], there is not enough evidence to whether more precise fat measures have stronger associations with CVD or S-CVD than traditionally used anthropometric measures of adiposity.

In a study by Menke et al., WC had stronger associations than total fat or proportion of total fat (measured by bio-impedance method) with several risk factors of atherosclerosis including; hypertension, diabetes, decreased HDL, increased triglyceride and increased HOMA-insulin resistance[6]. Another study did not find evidence to support ultra-sonographically

measured fat was better than anthropometric measurements in explaining variability in early atherosclerosis[23]. A German study found WC, after adjustment for BMI, to be superior to fat mass or percent body fat as measured by bio-impedance, in explaining variability in IMT and arterial stiffness[24]. In another study by Takami et al, intra-abdominal fat area measured with CT did not have stronger association with IMT compared to BMI or WC among Japanese males[25]. This clearly warrants further research to identify the best measure of total and regional adiposity that explains the most variance in S-CVD.

The Study of Women's Health Across the Nation (SWAN) and its ancillary study SWAN- Heart provide a unique opportunity to explore associations of total and regional adiposity measured with CT, DXA and anthropometry with subclinical measures of atherosclerosis among Caucasian (CA) and African American (AA) women transitioning through menopause. In this study we evaluated associations of total, trunk, arm and leg fat proportions (DXA measures); areas of whole adipose tissue (WAT), visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) (CT measures) and, weight, BMI, WC and WHR (anthropometric measures) with subclinical measures of atherosclerosis (carotid IMT and carotid AD) among women passing through menopausal transition.

4.3 Materials and Methods

The study of Women's Health Across the Nation (SWAN) is a multi-center, multi-ethnic, longitudinal epidemiologic study investigating the physical and psychosocial changes in middle-aged women during menopausal transition. Three thousand three hundred and two women were recruited at baseline at seven study sites. In addition to Caucasians (CA), each site recruited one other ethnic group, African American (AA) (Boston, MA; Chicago, IL; Detroit, MI; Pittsburgh, PA), Hispanic (Newark, NJ), Japanese (Los Angeles, CA) and Chinese (Oakland, CA). Main eligibility criteria for enrollment were: an intact uterus and at least one ovary, at least one menstrual period in previous 3-months, no use of reproductive hormones in previous 3-months and 42-52 years of age. Details of the study design and population have been already published elsewhere[26].

SWAN-Heart, an ancillary study of SWAN, designed to examine the risk factors correlated with sub-clinical CVD in AA and CA women in menopausal transition. Six hundred and eight women (participating in SWAN) were enrolled in SWAN-heart from Pittsburgh and Chicago. Participants were ineligible if they: a.) were taking medication for diabetes, hypertension or heart arrhythmias, b.) had a history of clinical atherosclerosis (MI, angina, intermittent claudication, cerebral ischemia or revascularization), c.) had had a hysterectomy and/or bilateral oophorectomy or d.) were taking female hormones.

The first round (baseline) of the ancillary study was conducted in conjunction with core SWAN annual interview at visits 4, 5, 6 and 7. Similarly, a follow-up SWAN heart study was conducted at SWAN annual follow-up visits 6, 7, 8 or 9. Women who did not participate at the baseline but did participate at the follow up of the ancillary study were also included in this analysis. Chicago and New Jersey sites did not participate in DXA protocol of SWAN. Since Pittsburgh is the only site participated in DXA protocol and had carotid as well as CT scan

components of SWAN heart study, the present study is based on the information provided by Pittsburgh participants only. The Institutional Review Board at the University of Pittsburgh approved the SWAN and SWAN-Heart studies.

4.3.1 Measurements

4.3.1.1 Subclinical atherosclerosis measurements

IMT and AD of CCA were measured on a duplex scanner, Toshiba SSA-270A (Toshiba, Shimoishigami, Japan). For IMT, images were taken from near and far walls of the distal common carotid artery, CC bifurcation and the first centimeter of the internal carotid artery. The AD was measured directly in common carotid as the distance from the adventitial-medial interface on near wall to the medial-adventitial interface on far wall at end-diastole. Artery Measurement System (AMS) computer software developed in Sweden (Gothenburg, Sweden) was used to quantify the digitized images. Reproducibility of measures was evaluated by replicating readings on 20 scans from these women. The intra-class correlation was 0.98 for IMT and 0.99 for adventitial diameter.

4.3.1.2 Body fat measurements

Weight, height and WC were obtained from SWAN annual visits corresponding to SWAN-Heart visits. Measurements were made in light clothing without shoes. Weight was measured to the nearest 0.1 kilograms (kg) in standing position and height was recorded to the nearest 0.1 centimeter (cm). WC was measured using metric measuring tape to the nearest 0.1 cm at the narrowest part of trunk (as seen from anterior aspect) above the umbilicus level. For WC measurement, participants wore either undergarment only or lightest possible cloth in standing position with their abdomen relaxed, arms at sides and feet together. BMI was later

calculated by dividing the weight in kg by height meter squared. Weekly calibration of portable scales and monthly calibration of study clinic based stationary scales were performed.

Following standard protocol, a certified technician performed whole body DXA scans on a Hologic QDR 2000 machine in fan-shaped-array mode. The Hologic software first categorizes each pixel in the scan as bone or soft tissue depending on tissue specific attenuation coefficients. Soft tissue is further categorized as fat and fat-free mass again using differential attenuation coefficients of fat and fat-free tissues. Participants were advised not to take any medication or supplement containing calcium for at least 24 hours before the scan. Participants reporting the use of x-ray with contrast such as, barium, IV contrast or any nuclear medicine for one week prior to the test date and those who tested positive on pregnancy test were not scanned. Participant were advised to remove all removable metal objects including rings, pins in ear or head, jewelry from their body. The coefficient of variation of fat measurement was 2.1%. Total leg and arm fat masses were obtained by adding left and right leg fat mass, and left and right arm fat mass respectively. Total body fat was calculated by adding trunk, total arm and total leg fats, excluding fat in the head. Instead of absolute fat mass, proportion of total and regional fat distributions were used in the regression models. Regional proportions of fat was calculated as percentage of total fat mass (e.g., proportion of trunk fat= trunk fat mass x 100/ total fat mass), while proportion of total fat was calculcated as percentage of total body weight (total fat mass x 100/ weight).

Images for VAT and WAT were obtained using Imatron C-150 Ultrafast CT scanner (Imatron, South San Francisco, CA) following standard protocol. CT produces an array of x-rays that pass through the body and are detected by the detectors located on the other side of the machine. Cross-sectional images were taken for each slice from different angles and later

combined to define each anatomic area. Six-millimeter images between lumbers 4 and 5 were digitized and later quantified by using software developed by Accuimage Diagnostic Corporation (San Francisco, CA). A region of interest line was drawn at the junction of subcutaneous fat and the abdominal wall muscles and was extended to around the body to the back muscles using trace function. The area of adipose tissue within the region of interest was determined using a pixel range of -190 to -30 Houndsfield Units. The intrabdominal cavity was outlined on the CT image and WAT and VAT areas were quantified by trained technician. The difference of WAT and VAT was interpreted as area of SAT.

4.3.1.3 Menopausal status

Women with at least one menstrual period in the past 3-months were categorized as premenopausal, if they had no change in regularity in the past 12 months and as early peri-menopausal if they had some change in regularity in the past 12 months. Similarly, women with at least one menstrual period in the past 12 months but none in the past 3-months were classified as late peri-, while those with no menses in the previous 12 months were categorized as postmenopausal. Pre- or early peri- or late peri- menopausal women reporting the use of birth control pills, estrogen, progestin or combination estrogen/ progestin in the past year were considered hormone therapy users. Women with hysterectomy with or without removal of ovary/ ovaries were classified as surgical menopause status. Women in hormone therapy and who had surgical menopause were excluded for this analysis. However, postmenopausal women using hormone therapy (n= 27) were not excluded from this analysis. Menopausal status was dichotomized by combining premenopausal and early peri-menopausal in 'earlier' group and, late peri- and post- menopausal into 'later' group, because the numbers of women in pre- (n= 20) and late peri- menopausal (n= 24) were very small.

4.3.1.4 Other covariates

Information on race, age, education level, smoking status and physical activities were collected at baseline SWAN visit. Women were categorized as current smoker versus past or never smoker based on self reported smoking status.

An average of two blood pressure readings on right arm as participant seated, following at least 5-minutes of rest were taken and for at least 30 minutes without smoking or ingesting caffeinated beverages. All readings were made to the nearest integer. Insulin, glucose, cholesterol, LDL, HDL, triglycerides levels were obtained from a fasting blood sample. All blood samples were frozen at -80^0 Celsius and shipped on dry ice to central laboratory (Medical Research Laboratory, Lexington, Kentucky), which is certified by the National Heart Lung and Blood Institute, Center for Disease Control [27]. Lipids were analyzed on EDTA treated plasma while total cholesterol and triglycerides were analyzed by enzymatic methods on Hitachi747 analyzer [28](Boehringer Mannheim Diagnostics, Indianapolis, IN). Serum insulin was measured using solid-phase radioimmunoassay (DPC Coat-A-Count, Los Angeles, CA) procedure, while plasma glucose was measured using a hexokinase-coupled reaction (Boehringer Mannheim Diagnostics, Indianapolis, IN).

4.3.2   Statistical methods

Normality for continuous variables and cell count for categorical variables were checked. Since IMT was not normally distributed, it was transformed into logarithmic scale and log of IMT was normal. Descriptive statistics were reported as mean ± SD for continuous variables and proportions for categorical variables. Ordinal logistic regression was used to determine the increasing or decreasing trend of independent variables and covariates with tertile of IMT. Conventional p-value of 0.05 was used to determine statistical significance.

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Data management and all analyses were done using SAS version 9.1, SAS Institute, Inc., North Carolina, USA. Graphs were created using Microsoft Excel 2003 (Microsoft Corporation, Redmond, WA, USA).

Associations of dependent and independent variables were first assessed using univariate correlations and unadjusted linear regressions. Predictors and covariates significantly associated with IMT and AD in univariate regression were grouped by similarity of the variables and further tested in linear regression models with stepwise criteria to identify the most significant variables. For example, DBP, SBP and heart rate were grouped in one group, while total cholesterol, LDL and triglycerides were selected as lipid group. However, HDL was not included in the lipid group because it's biological property is opposite from the rest of the lipid indicators. Similarly, glucose and insulin were grouped in another model. Variables found most significant in group models; LDL, SBP and insulin, and HDL were then used as a covariates in multivariate models.

To assess the impact of measures of adiposity on S-CVD, we first developed a base model including age, race, height and menopausal status only. Then each marker of adiposity was added to the base model separately. All measures of adiposity were further tested in fully adjusted separate multivariate linear regression models with base variables further adjusting for the identified covariates to assess if established risk factors explain more variability in IMT and AD. Final multivariate models for both outcome measures were adjusted for the effect of age, height, SBP, LDL, HDL, race, menopausal status and insulin level, except the model with BMI. Since BMI is a function of height and weight, the effect of height was not adjusted for BMI. In addition to above mentioned variables, proportions of trunk, arm and leg fat were further adjusted for corresponding proportions of lean mass to minimize the error due to DXA

assumption of constant hydration in the lean mass[17,29]. Multi-collinearity was assessed by variance inflation factor (VIF). Any two variables with VIF $\geq$ 2.0 was considered multi-collinear.

Prior studies have suggested racial differences in adiposity measurements and atherosclerosis. Hence, we also ran race specific fully adjusted regression models for explorative purpose only. Since the race specific analyses were lacking statistical power, only primary findings of the analyses were reported.

4.4 Results

Two hundred and fifty nine women out of 453 participating in core SWAN study agreed in writing to participate in the ancillary study. Of 259 women, 222 completed baseline carotid scans as well as CT scans (238 carotid and 222 CT scans), while 184 completed follow-up carotid and CT scan (212 carotid and 185 CT scans) for SWAN heart study (fig 4-1). Twenty one of 184 women in the follow-up who did not participate in the baseline component were also added to the baseline sample yielding total number of women to 243 with carotid and CT scans. Out of 243, seven women without DXA scans, 26 on exogenous hormone therapy (n= 15) or surgical menopause (n= 11) and one woman scanned in QDR 4500 machine instead of QDR 2000 were excluded from this analysis. Hence 209 women contribute to the current analysis.

Baseline characteristics by IMT tertiles are reported in table 4-1. Although 30.6% women in our study were AA, 54.3% of women in the highest tertile of IMT were AA (table 4-1). Mean values of LDL, insulin, SBP and distolic blood pressure were higher in higher IMT tertiles. However, the mean HDL values were lower in higher tertiles of IMT. The proporiton of women in later menopausal status (late peri- or postmenopausal) was the highest in the 3rd tertile of IMT.

Mean values of BMI, area of VAT and, amounts of leg and trunk fat masses for women in higher tertiles of IMT were larger than for women in lower tertile of IMT (figure 4-2).

4.4.1 Association with log IMT

In unadjusted correlation analyses, log IMT was positively correlated with WC, BMI, weight, DBP, SBP and areas of VAT, SAT and WAT. Similarly, insulin, proportion of total fat, proportion of trunk fat, proportion of arm fat were positively correlated with log IMT. In

contrast, SHBG, HDL and proporiton of leg fat were negatively correlated with log IMT (table 4-2).

In base regression model containing only age, race, height and menopausal status , African American women (p=0.02) were more likely to have thicker IMT (table 4-3a). After adding each anthropometric measure of adiposity separately to the base model, the base model plus WC explained the most variance (R^2=0.230), while the base model plus WHR explained the least (R^2=0.154) variance in log IMT. Similalry, among DXA adiposity measures, the base model plus proportion of leg fat explained the most variance (R^2=0.126) while the base model plus proportion of arm fat explained the least variance (R^2=0.096) in log IMT. Among adiposity measures from CT scan plus base model, VAT explained the most variance (R^2=0.160) and SAT expalined the least variance (R^2=0.124) in log IMT. Proportion of total fat was positively associated with log IMT (p=0.004), while the proportion of leg fat (p=0.006) was negatively associated with log IMT. However, the proportions of trunk (p=0.06) and arm (p=0.07) fats were positively associated with log IMT with borderline significance. Areas of VAT (p<0.001), WAT (p<0.001) and SAT (p=0.002) were positively associated with log IMT (table 4-3a).

After adding S-CVD's established risk factors; SBP, insulin, HDL and LDL to the base model, only WC (p=0.03) appeared to be independently associated, while WHR (p=0.05) was marginally associated with log IMT. Other measures of adiposity; proportions of total, trunk, arm and leg fats, and areas of VAT, SAT and WAT were not independently associated with log IMT after adjusting for established risk factors; SBP, insulin, HDL and LDL (table 4-4a).

When R^2 from regression models without established risk factors were compared, the model with WC (R^2=0.230) explained 96.7% and 30.3% more variances in log IMT than the models with proportion of trunk fat (R^2=0.117) and area of VAT (R^2=0.160), respectively (table

4-3a). Also, the model with area of VAT explained 37.1% more variance in log IMT than the model with proportion of trunk fat. After adjustment for SBP, insulin, HDL and LDL, the difference in variance explained by different measures of adiposity decreased substantially. Even after adjustment for established risk factors the model with WC appeared to be as good as the models with proportion of trunk fat or area of VAT , explaining 11% and 10% more variances in log IMT, respectively (table 4-4a).

In race-specific analyses, traditional risk factors explained most of the variance in IMT. Although adiposity measures explained very little additional variability in either the AA and CA analyses, more variance was explained by the fully adjusted regression model for AA (R^2 = 0.469) than in the fully adjusted regression model for CA (R^2 = 0.127). Again, WC appeared to be as good as proportion of trunk fat and VAT in explaining variances in IMT for both the races.

4.4.2 Association with AD

AD was positively correlated with weight, WC, BMI, DBP and SBP (table 4-2). Similarly, positive correlation of AD with areas of VAT, SAT and WAT, and proportions of total and trunk fat were observed. C-RP was also positively correlated with AD. In contrast, proportion of leg fat and HDL were negatively correlated with AD. Age (p=0.05) was positively correlated with AD while SHBG (p=0.08) was negatively correlated with AD with marginal significance.

In the base model, AA, taller women and women in later menopausal status were more likely to have larger AD (table 4-3b). The base model explained 14.6% variance in AD, as indicated by R^2. When anthropometric measures were added to the base model, weight in combination with other base variables explained the most variance (R^2=0.272) followed by WC,

BMI and WHR plus base variables. However, the differences of variances explained by weight and WC in combination with other covariates were minimal. Similarly among other parameters of adiposity, the regression model with area of VAT explained the most variance ($R^2=0.242$) followed by the models with proportions of total, leg and trunk fats, areas of WAT and SAT, and proportion of arm fat (table 4-3b).

In another phase of analyses, established risk factors of S-CVD; SBP, insulin, HDL and LDL were allowed into the minimally adjusted models. Weight, BMI, WC, WHR, proportions of total and trunk fats, and areas of VAT and WAT were found to be positively associated with AD, while proportion of leg fat was negatively associated with AD (table 4-4b).

When R^2s from different models without established risk factors were compared, the model with WC ($R^2=0.266$) explained 22.1% more variance than the model with proportion of trunk fat ($R^2=0.218$) and 8.9% more variance than the model with area of VAT ($R^2=0.242$). Area of VAT plus other covariates explained 11.3% more variance than proportion of trunk fat plus other other covariates (table 4-3b). After adding established risk factors (table 4-4b), the difference on variance explained by models with different measures of abdominal adiposity on AD decreased. However, the model with WC still explained more variance than the models with proportion of trunk fat by 7.5% and area of VAT by 4.5% in AD, while the models with area of VAT explained 2.7% higher variance than the model with proportion of trunk.

In a explorative race-specific analyses, the amount of variance explained by the fully adjusted regression model for AA ($R^2= 0.244$) was more than the variance explained by the fully adjusted regression model for CA ($R^2= 0.125$). The fully adjusted model with proportion of trunk fat explained slightly more variance in AD among AA ($R^2= 0.302$) compared to the variances explained by the models with WC ($R^2= 0.262$) or VAT ($R^2= 0.258$). However among CA, the

fully adjusted model with WC (R^2= 0.249) explained slightly more variance in AD than by the models with proporiton of trunk fat (R^2= 0.165) or VAT (R^2= 0.197).

4.5 Discussion

In present analysis, anthropometric measures of overall and regional adiposity and more precise measures of total and regional fat explained similar variances in log IMT and AD before and after adjusting for established risk factors of S-CVD including SBP, insulin, HDL and LDL. Our two primary findings are; a.) anthropometic meaures of overall adiposity, weight and BMI explained variances comparable to that explained by total body fat measured by proportion of total fat relative to weight and, b.) anthropometric measures of abdominal adiposity WC and WHR explained varainces comparable to the variances explained by measures of abdominal fat, assessed as proportion of trunk fat relative to total fat, VAT, SAT and WAT in log IMT and AD of carotid artery. Thus our findings provide no evidence that total or regional measures of fat distribution obtained by DXA or CT methodologies are better in explaining variances in atherosclerosis despite much higher cost, exposure to radiation and participant's burden.

This is the first study to our knowledge to compare three methods of adiposity measurement in the same study population in terms of S-CVD outcomes among women passing through menopausal transition.

Prior studies have compared measures of adiposity obtained by other methodologies such as bio-impedance, air displacement plethysmography with anthropometric measures. Our findings are consistent with these studies. A study utilizing bio-impedance technology also found anthropometric measures as strongly associated as fat measures with a number of disease including CVD and diabetes among elderly Britons[30]. In contrast, in a French study with 1,014 men and women, slightly older than our subjects, WC, fat mass and percent body fat (assessed by bio-impedance analysis) were associated with IMT[24]. In Czernichow's study, WC was also

associated with pulse wave velocity but not fat mass and percent body fat. A recent cross-sectional study from Germany did not find superiority of measures of body fatness (measured by air-displacement plethysmography) over anthropometric measurements in predicting metabolic risk related to obesity[31]. Furthermore, in a recent meta-analysis (n= 258,114) that evaluated the role of WC and WHR on cardiovascular events, de Koning et al confirmed that the risk of CVD events increases significantly with increase in WC and WHR[1].

Also, in a recent large (n= 12,608) study with US national representative sample Menke et al. found WC as a better predictor of risk factors of atheroscelrosis (hypertension, diabetes, decreased HDL, increased triglycerides and increased HOMA-IR) than bio-impedance fat measures or skinfolds measurements[6]. Menke et al., compared five measures of adiposity (WC, BMI, total body fat and proportion of total body fat), while we compared 11 measures (weight, WC, BMI, WHR, proportions of total fat, proportion of trunk fat, proporiton of leg fat, proportion of arm fat, VAT, SAT and WAT) of total and regional fat distribution obtained using either simple (anthropometric) or sophisticated technology (DXA and CT). Also, they used bio-impedance technology, whose applicability in real life has been less satisfactory due to underlying assumptions of unequal distribution of conductive material in different regions of human body and need of populaiton specific equations for analysis [32-35], whereas we used DXA and CT.

A Japanese study on men did not confirm the superiority of intra-abdominal fat (by CT) over BMI and WHR in predicting carotid IMT[25]. Although Takami et al's study was very close to our study in terms of outcome and exposure variables, we used DXA measures in addition to CT and anthropometric measures as they have used. In Takami et al's study, an independent association of WC with IMT lost statistical significance after adjusting for BMI, which may be

due to collinearity. Our study differed from Takami et al's study mainly in three aspects; first sample selection, they had only male while we analyzed only females; second we adjusted for more variables including SBP, insulin, LDL and height which they did not and third, we compared DXA adiposity measures in addition to CT and anthropometric measures.

Other studies have reported superiority of WC, WHR over more precisely measured abdominal fat indices in predicting S-CVD or its risk factors including insulin resistance, type II diabetes, hypertension, dyslipidemia[6,23,31,36-38], while other using abdominal fat or VAT have not found consistent results[25,39-41]. Stamatelopoulos et al., reported WC and WHR as stronger predictor of early atherosclerosis than ultrasonographically measured fat depots. However, the study had fewer subjects who were relatively younger than ours. In a small study (n= 32), Brook et al., found WHR as an independent predictor of vascular endothelial dysfunction, a marker of early atherosclerosis, indicating abdominal adiposity may play detrimental role on atherosclerosis[38]. In another relatively small (n= 54) study, Goodpaster et al. found subcutaneous abdominal fat as an independent correlate of insulin sensitivity even after controlling for visceral fat indicating the negative role of overall abdominal fat accruement rather than just VAT in risk factor of S-CVD. Area of subcutaneous adipose tissue but not VAT was significantly associated with carotid IMT in another small study reported by Lo et al[41].

Abdominal adiposity may influence atherosclerosis indirectly through several biological mechanisms including; dyslipidemia, hypertension, hyperglycemia, hyperinsuliminia, releasing a number of inflammatory markers[3,19,42-44] or directly by imparing endothelial function[38,45,46], flow-mediated dilation[23] increasing the availability of free fatty acids[47] or some other yet unknown mechanisms. Dyslipidemia, hypertension, hyperinsuliminia and inflammatory markers have been consistently shown to be an integral part of atherosclerosis[42,44]. Also, abdominal fat, mainly VAT

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is thought to be main obesity induced player associated with these risk factors because of it's active pro-lipolytic activity and higher ability to deliver free fatty acids and inflammatory markers directly to systemic circulation than other fat depots[18]. Most importantly, free fatty acids released from VAT have direct access to the liver which may impact negatively on hepatic insulin activity increasing the risk of atheroscelrosis[47]. Prior studies have also shown an inverse relationship of circulating adiponectin with abdominal adiposity suggesting increased abdominal adiposity reduces the amount of circulating adiponectin which is thought to be involved in anti-atherogenic activity[48-51].

Minimal change in R^2 after adjusting SBP, insulin, HDL and LDL in AD further suggests that certain markers of atherosclerosis (AD in present analysis) may be more sensitive than IMT to fat distribution. Majority of the prior studies evaluating the role of VAT on IMT[24,41,52] have not found positive association while others assessing the role of VAT on other markers of atherosclerosis such as pulse-wave velocity[4] have reported strong associations. These inconsistent findings may suggest that fat may have indirect role on IMT thickenning through other biological mechanism mainly SBP and insulin resistance. Our data also support this notion as the independent association of proportion of total fat, proportion of trunk fat, VAT, WAT with log IMT lost statistical significance after SBP was adjusted.

Main limitation of our study is, our inability to test whether the more variances explained by anthropometric measures in log IMT and AD were statistically significant or not as we were not aware of any statistical methods comparing R-squares obtained using different variables in regression models. Since our study population is women in menopausal transition, we would like to caution in applying our findings to male population or women in other stage of life. Race

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specific analyses were done for an explorative purpose and did not have enough statistical power. Also, present study is cross-sectional by design, the findings should not be inferred as casual.

In summary, our data suggests that abdominal adiposity has negative role on S-CVD. In present analysis, we found no evidence to support CT or DXA measured adiposity measures are better than traditionally used anthropometric measures of adiposity to explain variability in S-CVD. Measurements of WC require minimal training and is cost-effective. The instruments used is a simple non-elastic tape which is inexpenssive, easy to care and easy to carry that does not involve any exposure to radiation. Waist circumference a marker of abdominal adiposity may be more precise in determining the status of S-CVD than VAT or proportion of trunk fat obtained by expensive and highly sophisticated methods such as CT and DXA.
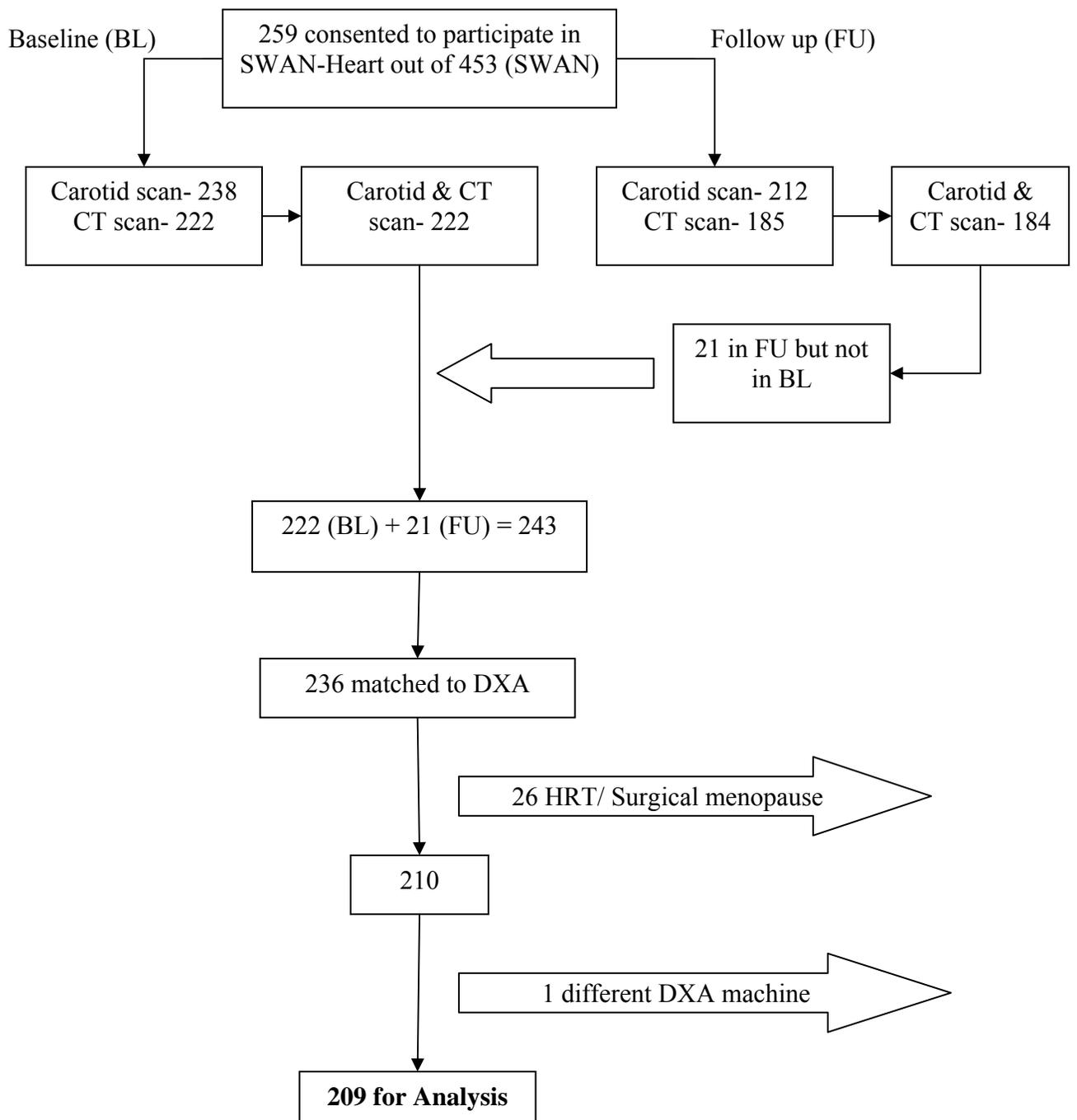
Figure 4-1. Flow chart of subject selection.

Table 4-1. Participant characteristics by IMT[#]

Characteristics	Tertiles of IMT (mm)				P-value*
	Combined N= 209	1st Tertile (0.53 – 0.65) N= 69	2nd Tertile (0.65 – 0.71) N= 70	3rd Tertile (0.72 – 1.18) N= 70	
African American (%)	30.6	17.4	28.6	54.3	**<0.001**
Age (years)	50.3 ± 2.6	49.6 ± 2.7	50.5 ± 2.5	50.6 ± 2.7	**0.030**
AD (mm)	6.6 ± 0.6	6.4 ± 0.5	6.6 ± 0.5	6.9 ± 0.6	**<0.001**
Cholesterol (mg/dl)	209.4 ± 39.4	207.4 ± 41.6	208.8 ± 35.4	211.9 ± 41.2	0.487
LDL (mg/dl)	126.7 ± 35.1	119.6 ± 35.6	128.0 ± 32.8	132.7 ± 36.0	**0.034**
HDL (mg/dl)	57.6 ± 14.2	60.5 ± 15.0	58.8 ± 13.8	53.9 ± 13.4	**0.009**
Triglycerides (mg/dl)	128.1± 107.5	135.2± 149.9	114.5 ± 73.6	131.5 ± 84.0	0.861
Glucose (mg/dl)	89.5 ± 19.1	88.8 ± 26.3	87.2 ± 14.9	92.1 ± 13.7	0.233
Insulin (uIU/ml)	11.4 ± 7.6	9.7 ± 7.0	10.5 ± 6.1	13.6 ± 8.6	**0.003**
CRP (mg/ l)	4.0 ± 4.7	4.0 ± 5.2	3.5 ± 4.1	4.5 ± 4.8	0.514
DBP (mmHg)	72.6 ± 8.7	69.7 ± 8.1	71.0 ± 7.9	77.1 ± 8.4	**<0.001**
SBP (mmHg)	114.5 ± 15.7	108.2 ± 12.5	113.0 ± 14.9	122.4 ± 16.3	**<0.001**
Menopausal status (%)					
Later	43.1	29.0	47.1	52.9	**0.005**
Smoking status (%)					
Yes	14.1	14.5	17.6	10.1	0.486
Education (%)					
<High school	0.5	1.5	0.0	0.0	
High school	21.4	13.0	20.6	30.4	
>High school	31.6	36.2	27.9	30.4	0.142
College	18.9	18.8	26.5	11.6	
Post graduate	27.7	30.4	25.0	27.5	

[#]- Values are expressed as proportions (categorical) and mean ± standard deviation (continuous).

*- P-values are based on unadjusted ordinal logistic regression.
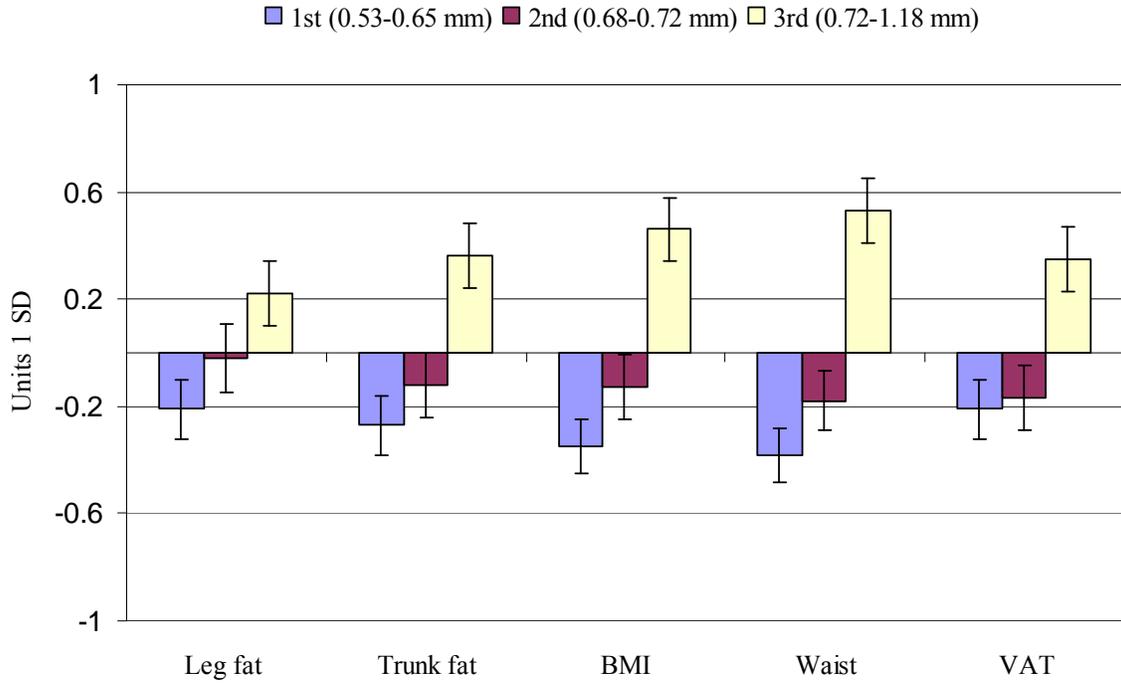
Fig. 4-2 Adiposity measures by tertile of IMT*

*- BMI, WC and trunk fat mass were significantly higher with increasing order of IMT tertile (all p-value <0.001). Leg fat mass (p= 0.014) and VAT (p= 0.001) were also significantly higher with increasing order of IMT tertile. The units are standardized to one standard deviation. The error bars represents ± 1 SD.

Table 4-2: Correlation of IMT and AD with predictors- (Spearman's)

Predictors	Subclinical CVD Markers n= 209			
	Log of IMT		AD	
	Corr coeff	p-value	Corr coeff	p-value
Age (years)	0.19	**0.006**	0.14	**0.054**
Weight (kg)	0.30	**<0.001**	0.40	**<0.001**
BMI (kg/m^2)	0.34	**<0.001**	0.36	**<0.001**
WC (cm)	0.38	**<0.001**	0.37	**<0.001**
Total fat (%)	0.23	**0.001**	0.32	**<0.001**
Trunk fat (%)	0.16	**0.018**	0.24	**<0.001**
Arm fat (%)	0.18	**0.009**	0.07	0.354
Leg fat (%)	-0.20	**0.004**	-0.24	**<0.001**
VAT (cm^2)	0.25	**<0.001**	0.34	**<0.001**
SAT (cm^2)	0.23	**<0.001**	0.28	**<0.001**
WAT (cm^2)	0.26	**<0.001**	0.32	**<0.001**
Cholesterol (mg/dl)	0.05	0.470	0.03	0.658
LDL (mg/dl)	0.16	**0.030**	0.05	0.462
HDL (mg/dl)	-0.22	**0.002**	-0.15	**0.045**
Triglyceride (mg/dl)	0.10	0.184	0.06	0.400
Insulin (uIU/ml)	0.25	**<0.001**	0.34	**<0.001**
Glucose (mg/dl)	0.18	**0.014**	0.21	**0.004**
C-RP (mg/l)	0.15	**0.037**	0.27	**<0.001**
DBP (mmHg)	0.37	**<0.001**	0.28	**<0.001**
SBP (mmHg)	0.40	**<0.001**	0.33	**<0.001**
Estrogen	-0.20	**0.004**	-0.19	**0.008**
SHBG	-0.26	**<0.001**	-0.17	**0.018**
DHEAS	-0.16	**0.020**	-0.09	0.202
FSH	0.13	**0.059**	0.11	0.125

IMT- Intima media thickness

AD- Adventitial diameter.

Table 4-3a: Base multivariate linear regression models (IMT)

Variables	Intima media thickness		
	R^2	β (SE)	p-value
Base model			
Age (yrs)		0.007 (0.004)	*0.07*
AA vs CA	0.081	0.045 (0.019)	**0.02**
Height per 10 cm		-0.007 (0.014)	0.60
Later Status vs Earlier		0.029 (0.021)	0.17
*Adiposity & fat measures**			
Weight (kg)	0.194	0.003 (0.0005)	**<0.001**
BMI (kg/m^2)$^{\#}$	0.200	0.007 (0.001)	**<0.001**
WHR	0.154	0.502 (0.123)	**<0.001**
Waist (cm)	0.230	0.004 (0.0006)	**<0.001**
Total fat (%)	0.118	0.003 (0.001)	**0.004**
Trunk fat (%)	0.117	0.002 (0.001)	0.06
Arm fat (%)	0.096	0.009 (0.005)	0.07
Leg fat (%)	0.126	-0.002 (0.001)	**0.03**
VAT (cm^2)	0.160	0.0005 (0.0001)	**<0.001**
SAT (cm^2)	0.124	0.0002 (0.00006)	**0.002**
Whole fat (cm^2)	0.145	0.0002 (0.00004)	**<0.001**

IMT- Intima media thickness

*- Adjusted for age, race, height and menopausal status.

$^{\#}$- BMI was not adjusted for height.

Each adiposity or fat measures were adjusted for all base variables and proportions of trunk fat, arm fat and leg fat were also adjusted for respective proportions lean masses.

β- parameter estimates; SE- standard error of parameter estimates.

Table 4-3b: Base multivariate linear regression models (AD)

Variables	Adventitial diameter		
	R^2	β (SE)	p-value
Base model			
Age (yrs)		0.019 (0.018)	0.29
AA vs CA	0.146	0.318 (0.084)	**<0.001**
Height per 10 cm		0.162 (0.061)	**0.01**
Later Status vs Earlier		0.223 (0.093)	**0.02**
Adiposity & fat measures[*]			
Weight (kg)	0.272	0.013 (0.002)	**<0.001**
BMI (kg/m^2)[#]	0.235	0.034 (0.006)	**<0.001**
WHR	0.223	2.65 (0.560)	**<0.001**
Waist (cm)	0.266	0.015 (0.003)	**<0.001**
Total fat (%)	0.219	0.020 (0.005)	**<0.001**
Trunk fat (%)	0.218	0.020 (0.005)	**<0.001**
Arm fat (%)	0.155	0.030 (0.021)	0.16
Leg fat (%)	0.218	-0.019 (0.005)	**<0.001**
VAT (cm^2)	0.242	0.003 (0.0006)	**<0.001**
SAT (cm^2)	0.195	0.0009 (0.0002)	**<0.001**
Whole fat (cm^2)	0.219	0.0008 (0.0002)	**<0.001**

AD- Adventitial diameter

*- Adjusted for age, race, height and menopausal status.

#- BMI was not adjusted for height.

Each adiposity or fat measures were adjusted for all base variables and proportions of trunk fat, arm fat and leg fat were also adjusted for respective proportions lean masses.

β- parameter estimates; SE- standard error of parameter estimates

Table 4-4a: Multivariate fully adjusted linear regression models (IMT)

Variables	Intima media thickness		
	R^2	β (SE)	p-value
Base model			
Age (yrs)		0.003 (0.004)	0.37
AA vs CA		0.015 (0.020)	0.46
Height per 10 cm		-0.007 (0.014)	0.59
Later Status vs Earlier	0.224	0.015 (0.021)	0.47
LDL per 10 mg/dl		0.004 (0.026)	0.17
HDL per 5 mg/dl		-0.007 (0.003)	**0.04**
Insulin uIU/ml		0.002 (0.001)	0.23
SBP per 10 mmHg		0.024 (0.006)	**<0.001**
Adiposity & fat measures[*]			
Weight (kg)	0.230	0.0008 (0.0007)	0.25
BMI (kg/m^2)[#]	0.230	0.002 (0.002)	0.21
WHR	0.247	0.286 (0.146)	**0.05**
Waist (cm)	0.252	0.002 (0.0008)	**0.03**
Total fat (%)	0.224	-0.0002 (0.001)	0.85
Trunk fat (%)	0.227	-0.001 (0.002)	0.40
Arm fat (%)	0.229	0.004 (0.005)	0.40
Leg fat (%)	0.226	0.0009 (0.001)	0.54
VAT (cm^2)	0.227	0.0001 (0.0002)	0.43
SAT (cm^2)	0.224	0.000 (0.00006)	0.99
Whole fat (cm^2)	0.224	0.00001 (0.00005)	0.80

IMT- Intima media thickness

*- Adjusted for age, race, height, menopausal status, SBP, insulin, HDL and LDL.

#- BMI not adjusted for height.

Each adiposity or fat measures were adjusted for all base variables and proportions of trunk fat, arm fat and leg fat were also adjusted for respective proportions lean masses.

β- parameter estimates; SE- standard error of parameter estimates

Table 4-4b: Multivariate fully adjusted linear regression models (AD)

Variables	Adventitial diameter		
	R^2	β (SE)	p-value
Base model			
Age (yrs)		0.007 (0.019)	0.72
AA vs CA		0.233 (0.098)	**0.02**
Height per 10 cm		0.146 (0.065)	**0.03**
Later Status vs Earlier	0.224	0.232 (0.010)	**0.03**
LDL per 10 mg/dl		0.0008 (0.013)	0.95
HDL per 5 mg/dl		-0.008 (0.017)	0.61
Insulin uIU/ml		0.008 (0.006)	0.21
SBP per 10 mmHg		0.081 (0.030)	**0.008**
Adiposity & fat measures[*]			
Weight (kg)	0.275	0.011 (0.003)	**<0.001**
BMI (kg/m^2)[#]	0.243	0.027 (0.009)	**0.003**
WHR	0.258	2.364 (0.146)	**0.001**
Waist (cm)	0.270	0.014 (0.004)	**<0.001**
Total fat (%)	0.248	0.014 (0.006)	**0.02**
Trunk fat (%)	0.251	0.016 (0.007)	**0.03**
Arm fat (%)	0.225	0.014 (0.025)	0.58
Leg fat (%)	0.253	-0.016 (0.007)	**0.02**
VAT (cm^2)	0.258	0.002 (0.0008)	**0.007**
SAT (cm^2)	0.235	0.0005 (0.0003)	0.12
Whole fat (cm^2)	0.244	0.0005 (0.0002)	**0.04**

AD- Adventitial diameter.

*- Adjusted for traditional risk factors; SBP, insulin, HDL and LDL.

#- BMI not adjusted for height.

Each adiposity or fat measures were adjusted for all base variables and proportions of trunk fat, arm fat and leg fat were also adjusted for respective proportions lean masses.

β- parameter estimates; SE- standard error of parameter estimates

5.0 Fat partition and aortic stiffness- a cross sectional study from the Study of Women's Health Across the Nation (SWAN)

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(Manuscript in preparation)

5.1   Abstract

Objective

To compare the variances explained by three different methods of total and regional adiposity measurements on carotid femoral pulse wave velocity (cfPWV) among women in menopausal transition.

Methods

Carotid femoral pulse wave velocity (cfPWV) was measured in the right common carotid and right femoral artery in 209 healthy Caucasian (69.44%) and African American (30.6%) women with mean age 50.6 ± 2.6 years, enrolled in the Study of Women's Health Across the Nation- Heart (SWAN-Heart). Multivariate linear regression was used to assess the variances, as indicated by R^2, explained by regression models with weight, body mass index (BMI), waist circumference (WC), waist to hip ratio (WHR); dual-energy x-ray absorptiometry (DXA) measured proportions of total, trunk, leg and arm fat mass, and computed tomography measured areas of visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and whole adipose tissue (WAT).

Result

In a separate multivariate regression models, BMI, weight, WC, proportion of total fat as measured by DXA and areas of VAT, SAT and WAT as measured by CT were each positively associated with log cfPWV even after adjusting for age, race, height and menopausal status . After further adjustment for systolic blood pressure, insulin and C-reactive protein, only the proportion of total fat relative to the body weight remained positively associated with log cfPWV. Models including above mentioned covariates and weight (R^2= 0.102), BMI (R^2=

0.101), WHR ($R^2= 0.087$), WC ($R^2= 0.095$), proportions of total ($R^2= 0.110$) and trunk ($R^2= 0.099$) fat, areas of VAT ($R^2= 0.103$), SAT ($R^2= 0.093$) and WAT ($R^2= 0.098$) explained comparable variance in cfPWV.

Conclusion

These findings indicate that proportion of total body fat has negative impact on arterial stiffness even after accounting for the effects of other potential confounder and risk factors. In addition, anthropometric measurements of adiposity explained similar variances to the variances explained by more precisely measured fat measures using DXA or CT methodologies in arterial stiffness. Our findings encourage the use of anthropometric measures in evaluating the role of adiposity on arterial stiffness, because the methodology requires minimal training, is cost-effective and does not involve any risk to the study subjects.

5.2   Introduction

Arterial stiffness is characterized by the loss of arterial elasticity which limits the artery's ability to accommodate the volume ejected by the left ventricle eventually leading to increased pulse pressure and isolated systolic hypertension[103]. Arterial stiffness is an important determinant of systolic blood pressure[194,195] and, in the presence of hypertension, left ventricular hypertrophy.[196] For that reason, arterial stiffness is considered a reliable indicator of vascular aging. Increased arterial stiffness is a risk factor of cardiovascular and all cause mortality[97,99,197,198], particularly with the presence of co-morbid conditions such as end-stage renal disease[99] and hypertension[199-201]. Prior studies have reported positive associations of aortic stiffness with cardiovascular disease[97,101,202,203], stroke[204] and renal disease[205].

Pulse wave velocity (PWV) is a measure of arterial stiffness that can be measured centrally (carotid-femoral artery) or peripherally[206] (carotid, brachial or femoral arteries). Carotid-femoral pulse wave velocity (cfPWV) has been reported as a predictor of composite cardiovascular end points (cardiovascular mortality, coronary heart disease and stroke) even after adjusting for the effect of cardiovascular disease (CVD) risk factors including age, BMI and smoking[100]. cfPWV is a non-invasive, reproducible and well accepted marker of central arterial stiffness[100,207] and is considered the 'gold standard' measurement of arterial stiffness[206]. Faster cfPWV indicates a stiffer artery and vice versa [101,104]. Additionally, hypertension, heart rate, glucose levels, triglyceride, smoking have been found to be positively correlated with arterial stiffness, measured as cfPWV[102,208-210]. Furthermore, a wide range of adiposity measures including BMI[46,209,211], WC[102,158], total body fat[212], trunk fat [3,213], VAT[139] have been reported to have positive associations with arterial stiffness.

Due to its increased lipolytic activity and inflammatory response, abdominal adiposity, particularly VAT, is now identified as having more detrimental effects on clinical and subclinical CVD than general adiposity, total body fat or peripheral fat [5,26,143]. Hence, it may be plausible to hypothesize that more precise measures of abdominal adiposity are better predictors of arterial stiffness than anthropometric adiposity measures such as WC. A study by Sutton-Tyrrell et al reported a positive association of VAT with increased aortic stiffness as indicated by a higher cfPWV [139] in older adults. However, such relationship has not been examined among women passing through menopausal transition. Also, there are few reports comparing the role of different measures of total and regional adiposity including BMI, total fat, trunk fat, VAT and WC on pulse wave velocity.

In the present study, we compared the variances explained by three different methods of total and regional adiposity measurement on carotid femoral pulse wave velocity. We included the following adiposity measurements; i) total, trunk, arm and leg fat proportions obtained by DXA methodology, ii) areas of WAT, VAT and SAT measured by CT methodology and iii) weight, BMI, WC and WHR measured by anthropometric measurements.

5.3 Methods

5.3.1 Study population

The study of Women's Health Across the Nation (SWAN) is a multi-center, multi-ethnic, longitudinal epidemiological study studying the physical and psychosocial changes in women experiencing menopausal transition. Three thousand three hundred two women were recruited at seven study sites. Each study site recruited Caucasian (CA) and one other ethnic group; African American (AA) (Boston, MA; Chicago, IL; Detroit, MI; Pittsburgh, PA), Hispanic (Newark, NJ), Japanese (Los Angeles, CA) and Chinese (Oakland, CA). Main eligibility criteria for enrollment were: a.) an intact uterus and at least one ovary, b.) at least one menstrual period in previous 3-months, c.) no use of reproductive hormones in previous 3-months and d.) 42-52 years of age. Details of the study design and population have been published previously [156].

SWAN- Heart is an ancillary study of SWAN, designed to examine risk factors for sub-clinical CVD in AA and CA women in menopausal transition. Six hundred eight SWAN participants were enrolled in SWAN-Heart at Pittsburgh and Chicago. SWAN participants were eligible for SWAN-Heart study unless they: a.) were taking female hormones or medication for diabetes, hypertension or heart arrhythmias, b.) had a history of clinical atherosclerosis (MI, angina, intermittent claudication, cerebral ischemia or revascularization) or c.) had had a hysterectomy and/or bilateral oophorectomy.

A single baseline SWAN- Heart study was conducted for each participant in conjunction with her core SWAN interview at annual follow-up visits 4, 5, 6 or 7. Similarly, a single follow-up SWAN- Heart study was conducted in conjunction with SWAN annual interview at visits 6, 7, 8 or 9. Five SWAN sites including Pittsburgh but not Chicago performed annual DXA scans. Therefore, present study is based on data obtained from Pittsburgh only. Both SWAN and

SWAN-Heart studies were approved by the Institutional Review Board of the University of Pittsburgh.

5.3.2 Analytic Sample

Figure 5-1 illustrates the details of SWAN-Heart participants included in the present analysis. Two hundred fifty nine women out of 453 participating in the core SWAN study at Pittsburgh consented to participate in the SWAN-Heart study. Of 259 women, 222 completed baseline cfPWV as well as CT protocols (238 cfPWV and 222 CT scans), while 184 completed follow-up cfPWV and CT (212 cfPWV and 185 CT scans) protocols for SWAN heart study. All 222 with concurrent cfPWV and CT data at baseline plus 21 women with concurrent cfPWV and CT data at follow-up were eligible for the current study. Of these 243 women, seven did not have DXA scans and 26 were either on exogenous hormone therapy or had undergone surgical menopause making them ineligible for this analysis. Of the remaining 210 women, one was scanned on QDR 4500 machine while the rest were scanned on QDR 2000 and thus she has been excluded from this analysis to minimize the noise in the data due to two different models of DXA machine. Hence this analysis is based on the information obtained from 209 women.

5.3.3 Measurements

5.3.3.1 Body fat measurements

Anthropometric measurements and DXA whole body scans were obtained during the annual SWAN visits corresponding to the SWAN-Heart visits. Weight, height and WC were measured in standing position. Weight was measured to the nearest 0.1 kilograms in light clothing. Height was measured on a flat surface and recorded to the nearest 0.1 centimeter (cm). WC was measured in undergarments or lightest possible clothing with the abdomen relaxed,

arms at sides and feet together. WC was measured using metric measuring tape at the narrowest part of torso, as seen from anterior aspect, which is normally above the umbilicus level. WC measurements were recorded to the nearest 0.1 cm. BMI was calculated as the ratio of weight in kilogram to height in meter squared. Weekly calibration of portable scales and monthly calibration of study clinic based stationary scales were performed.

DXA distinguishes bone, fat and lean masses based on energy attenuation differences. Following a standard protocol, whole body DXA scans were performed by a trained and certified technician on a Hologic QDR 2000 system in fan-shaped-array mode. Participants were asked not to take any medication or supplement containing calcium at least 24 hours before the scan. Subjects who used contrast for an x-ray or used any nuclear medicine within one week prior to the scan date and those found positive on pregnancy test were not scanned. Participants were advised to remove all removable metal objects from the body. The coefficient of variation of adiposity measurement was 2.1%. Total leg and arm fat masses were obtained by adding left and right leg fat mass, and left and right arm fat mass respectively. Total body fat was calculated by adding trunk, total arm and total leg fats, excluding fat in the head. Since there are some technical problems associated with measuring fat in the head, we excluded head fat from the total body fat measurement. We used proportion of total and regional fat distributions in the regression models, instead of absolute fat masses, because the proportion of fat provides a better measure of regional fat distribution than absolute fat mass. Proportion of fat in a specific anatomical region was calculated as percentage of total body fat mass (e.g., proportion of trunk fat= trunk fat mass x 100/ total body fat mass), while proportion of total fat was calculcated as percentage of total body weight (total body fat mass x 100/ total body weight).

CT scans were performed at each SWAN-Heart visits using Imatron C-150 Ultrafast CT scanner (Imatron, South San Francisco, CA). Arrays of x-rays from the source passed through the body and were detected by the detectors located on the other side of the machine. Participants were asked to hold their breath during the scan. With 200 milliseconds of exposure time, cross-sectional images were taken for each 6-milimeter slice between lumbers 4 and 5 from different angles and later combined to define an anatomic area. Scan data were saved on optical disc and were quantified using software developed by Accuimage Diagnostic Corporation (San Francisco, CA) at Preventive Heart Care Center (Pittsburgh, PA). Using trace function a region of interest (ROI) was identified at the junction of subcutaneous fat and the abdominal wall muscles and was extended around the body to the back muscles. The area of adipose tissue within ROI was determined using a pixel range of -190 to -30 Houndsfield Units. A trained and certified technician identified the intrabdominal cavity and quantified VAT and WAT. The difference of WAT and VAT was considered as SAT.

5.3.3.2 Aortic pulse wave velocity measurements

Aortic pulse wave velocity was measured from the right common carotid and right femoral artery taking simultaneous recordings of the arterial flow waves on unidirectional transcutaneous Doppler Flowmeter, model number 810-A (Parks Medical Electronics Inc, Aloha, OR). Before cfPWV measurement each participant rested in supine position for 30 minutes. For each subject, three separate data collection runs, each obtaining a minimum of 10 pairs of simultaneously recorded flow waves were performed. An average of the flow waves was obtained and the time from the R wave of the electrocardiogram to the foot of the pressure wave was determined while scoring. The distance between the carotid and femoral sampling sites was

measured using a standard measuring tape over the body surface and divided by the time differential in the arrival of the pressure wave.

5.3.3.3 Menopausal status

Self reported menstrual history was used to determine the menopausal status of the study subjects. Women with at least one menstrual period in the past 3-months with no change in cycle regularity in the past 12 months were categorized as premenopausal, while women with some change in cycle regularity over the previous 12 months were classified as early perimenopausal. Late perimenopausal status was assigned to those with no menstrual period within the past 3-months, but one in the past 12 months. Postmenopausal status was assigned to women who did not have menstrual period within the past 12 months. Women reporting the use of external female hormones including birth control pills, estrogen, estrogen injection/ patch, combination estrogen/ progestin, or progestin pills in the past year were categorized as hormone therapy users. Women with hysterectomy with or without removal of one or both ovaries were grouped in surgical menopause category. Women who initiated hormone therapy use before experiencing 12 months without a menstrual period and women who had surgical menopause were excluded from current analysis. Because of the small number of women in premenopausal (n= 20) and late perimenopausal (n= 24) categories, menopausal status was dichotomized by combining premenopausal and early perimenopausal in one group and late peri- and postmenopausal in another.

5.3.3.4 Other covariates

Other covariates including race, age, education and smoking status were collected at the initial SWAN visit. Smoking status was dichotomized as current smoker versus past smokers or never smokers.

Following a 5-minute rest, two blood pressure readings were taken in right arm with the participants seated. The average of these two measurements was used for these analyses. Participants were required not to smoke or drink any caffeinated beverages 30 minutes prior to the blood pressure measurements.

As required by the SWAN protocol, a fasting blood sample was obtained and analyzed for insulin, glucose, cholesterol, LDL, HDL, triglycerides. Blood samples were stored at 4^0 Celsius until plasma and serum were separated and then frozen at negative 80^0 Celsius. Frozen blood samples were shipped to Medical Research Laboratories (Lexington, Kentucky), a certified [178] facility. Hitachi 747 analyzer was used to analyze triglycerides and total cholesterol and heparin-2M manganese chloride was used to isolate high density lipoprotein (HDL). Low density lipoprotein (LDL) was estimated using Friedewald equation[214]. Plasma insulin was analyzed using solid-phase radioimmunoassay procedure (DPC Coat-A-Count, Lost Angeles, CA). Plasma glucose was estimated using hexokinase-coupled reaction (Boehringer Manheim, Indianapolis, IN). C-reactive protein (CRP) was measured using the Behring Nephelometer II (hs-CRP on BN 100, Dade-Behring, Marburg, Germany).

5.3.4 Statistical methods

Data were managed and analyzed using SAS version 9.1 (SAS Institute, Inc., North Carolina, USA). Graphs were created using Microsoft Excel 2003 (Microsoft Corporation, Redmond, WA, USA).

Normality assumptions for continuous variables and cell count for categorical variables were checked. Since cfPWV was not normally distributed, it was transformed into logarithmic scale and log of cfPWV appeared to be normal. Descriptive statistics for continuous variables were illustrated as mean ± standard deviation, while that for categorical variables were expressed

as proportions. The increasing or decreasing trend of independent variables and covariates with cfPWV tertiles were evaluated using ordinal logistic regression. Conventional p-value of 0.05 was used to judge statistical significance.

Potential confounders/ covariates associated with both independent and dependent variables of interest were identified from available literatures. Associations of adiposity markers and covariates with cfPWV were assessed with unadjusted correlation analysis and univariate linear regression. Variables that were either statistically signficant (p<0.05) or marginally significant (0.05 ≤ p < 0.10) with cfPWV in univariate regression models were grouped by similarity of the variables and further tested in linear regression model using stepwise criteria. For example, diastolic blood pressure (DBP), systolic blood pressure (SBP), pulse rate and pulse pressure were grouped in one model, while glucose and insulin were included in another model. The most significant variables in each group model (SBP and insulin) were retained for the final multivaratie modelling. Since CRP was significantly associated with cfPWV in univariate regression and we did not have other markers of inflammation, it was considered for inclusion in the multivariate model. None of the lipid markers were associated with cfPWV and thus were not considered for inclusion in multivariate model.

Since age, race and menopausal status are non-modifiable risk factors of cfPWV and the total and regional fat distribution depends on height, we forced them into the multivariate regression model. An attempt was made to assess the true impact of different measures of adiposity on cfPWV by first developing a base model including age, race, height and menopausal status only. Then each measure of adiposity was added to the base model separately. Each measure of adiposity was further tested in expanded separate multivariate linear regression models controling for the identified covariates; SBP, insulin and CRP, in addition to above

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mentioned base model variables. This would let us to assess if established risk factors explain additional variance in cfPWV than that explained by base variables plus adiposity markers. Since BMI is a function of height and weight, the height was not included in the model with BMI. Regional proportions of fat were adjusted for corresponding proportions of lean mass in addition to above mentioned variables to minimize the error due to DXA assumption of constant hydration in the lean mass[18,215]. Multi-collinearity was assessed by variance inflation factor (VIF). Any two variables with VIF ≥ 2.0 was considered multi-collinear.

Since existing liturature suggest racial difference in adiposity measurements as well as in arterial stiffness, we also ran race specific fully adjusted regression models for explorative purpose only. Since the race specific analyses were lacking statistical power, only primary findings of the analyses were reported.

5.4   Results

Table 5-1 illustrates characteristics of the study sample by the tertile of cfPWV. Mean age of participants was 50.3 years. One in five (20.9%) women in the lowest tertile of cfPWV were AA, while almost one-half of the women in the highest tertile were AA. There was a tendency of women in a higher tertile of cfPWV to have higher BMI, pulse pressure, CRP, insulin, DBP and SBP. In contrast, pulse rate, total cholesterol, LDL, HDL, triglycerides, menopausal status and smoking status were not statistically different by tertile of cfPWV. In unadjusted ordinal logistic regression, women with higher mean values of BMI ($p \leq 0.001$), WC ($p \leq 0.001$), absolute values of trunk fat mass ($p \leq 0.001$) and leg fat mass ($p = 0.001$), and area of VAT ($p = 0.005$) were more likely to be in a higher tertile of cfPWV (figure 2).

In univariate Spearman's correlation analysis (table 5-2), log cfPWV was positively correlated with weight, BMI, WC, WHR, proportions of total and trunk fat, and areas of VAT, SAT and WAT. Similarly, pulse pressure, DBP, SBP, CRP and insulin were also positively correlated with cfPWV. In contrast, HDL and proportion of leg fat were negatively correlated with cfPWV. Among hormonal variables, DHEAS was negatively correlated with cfPWV with marginal significance.

In the multivariate base model, AA women were more likely to have higher values of log cfPWV compared to CA women after adjusting for age, height and menopausal status (table 5-3). In separate regression models including the base variables and one adiposity measures at a time, BMI ($R^2 = 0.109$), weight ($R^2 = 0.107$) and WC ($R^2 = 0.095$) were all positively associated with cfPWV and the models explained comparable variability in log cfPWV. Among DXA parameters, only proportion of total fat ($R^2 = 0.103$) was positively associated with log cfPWV.

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Areas of VAT (R^2= 0.095), SAT (R^2= 0.082) and WAT (R^2= 0.092) were positively associated with log cfPWV.

The base model was then expanded by adding SBP, insulin and CRP which were identified as risk factors of cfPWV. In this expanded base model, race was the only variable positively associated with log cfPWV (table 5-4). Then each measure of total and regional adiposity was entered separately into the expanded model. Among measures of adiposity, proportion of total fat (p= 0.042) was positively associated with log cfPWV, while BMI (p= 0.086) and area of VAT (p= 0.090) were marginally associated with cfPWV. Variability on cfPWV explained by the expanded models that included weight (R^2=0.102), BMI (R^2=0.101), WC (R^2=0.095), proportions of total (R^2= 0.110) and trunk fat (R^2= 0.099), areas of VAT (R^2= 0.103), SAT (R^2= 0.093) and WAT (R^2= 0.098) were comparable. Although models including proportions of arm fat (R^2=0.149) and leg fat (R^2=0.143) explained relatively higher variance on log cfPWV than other adiposity markers, neither proportion of arm fat nor the proportion of leg fat were associated with cfPWV after adjusting age, race, height, menopausal status, SBP, insulin and CRP. The higher variances in cfPWV explained by the models that included proportions of leg and arm fat were due to the proportions of leg and arm lean masses, which were also included in the models. In fact, proportion of leg lean mass (p= 0.003) was positively and proportion of arm lean mass (p= 0.001) was negatively associated with cfPWV.

The expanded base model explained 58.2% more variance in cfPWV than the base model without SBP, insulin and CRP (table 5-5). However, for the individual models that included the various adiposity measures, in most cases the addition of SBP, insulin and CRP only increased the explained variance minimally (<10%); exceptions were the models for WHR, arm fat and area of SAT where the variance increased by 42.6%, 63.7% and 13.4% respectively.

We also ran the expanded multivariate models excluding women (n=19) with CRP values ≥ 10 mg/l, because higher CRP value (≥ 10 mg/l) may indicate acute infection which may be confounding the relationship of adiposity markers and cfPWV. Since the variances (obtained after excluding women with CRP ≥ 10mg/l) explained by the models with weight, BMI, WC, and area of VAT were similar to that obtained without excluding women with CRP ≥ 10mg/l, we included these women in our primary analysis. However, we should note that in secondary analysis excluding women with CRP values ≥ 10mg/l, the relationship of weight, BMI, WC, and area of VAT with cfPWV did become statistically significant (data not shown).

In race specific analysis, although fully adjusted regression models explained more variances in AA ($R^2= 0.258$) than in CA ($R^2= 0.014$); the variances explained by WC (R^2s 0.021 for CA and 0.282 for AA), proportion of trunk fat (R^2s 0.047 for CA and 0.296 for AA) and VAT (R^2s 0.057 for CA and 0.262 for AA) were comparable within each race. VAT (p= 0.033) and proportion of total fat (p= 0.028) were positively associated with cfPWV among CA but not among AA (p-values 0.518 for VAT and 0.717 for proportion of total fat). Among traditional risk factors, CRP appeared to be important for AA (p= 0.006) but not for CA (p= 0.964).

5.5 Discussion

This study compares the variance explained by 11 different measures of total or regional adiposity, measured by three different methods on arterial stiffness among women in menopausal transition. In this analysis, cfPWV variability (as indicated by R^2) explained by the regression models with anthropometric measures (weight, BMI and WC), DXA measures (proportions of total fat relative to body weight and proportion of trunk fat relative to total fat) and CT measures (areas of VAT, SAT and WAT) were comparable after adjusting for age, race, menopausal status, height, SBP, insulin and CRP. Although in expanded model, base variables plus proportion of both leg and arm fat relative to total fat explained slightly more variability on cfPWV than other expanded models with measures of total and regional adiposity, associations were not statistically significant. In fact, the higher variance explained by the models with proportions of leg and arm fat was because of the proportions of the respective lean masses. Only proportion of the total fat was positively and significantly associated with cfPWV after adjusting for above mentioned covariates in the expanded model.

While our main focus was to compare the variances explained by different measures of adiposity in cfPWV, the observed positive and significant relationship between proportion of total fat relative to body weight and cfPWV clearly adds to the body of scientific evidence showing that increased fat accumulation in the body poses deleterious effect on arterial elasticity.

Our findings are consistent with prior studies evaluating the role of BMI, weight, WC and area of VAT on arterial stiffness. From the Framingham Heart Study, Mitchell et al reported a positive association of BMI with abnormal aortic stiffness after controlling for age [209]. However, they did not examine similar relationship between WC and arterial stiffness. Our study differs from Mitchell's study in the age of participants (mean age 62 yrs in Mitchell et al's study vs 50.3

yrs in our study) and they included both males and females while our sample had only females .

A study by Sutton-Tyrrell et al reported a strong positive association (p<0.001) of area of VAT

and cfPWV among older adults (mean age 74 yrs) [139]. Similarly, Mackey et al found a significant

positive association between WC and cfPWV among elderly adults (mean age 78.2 yrs),

however, in gender specific analysis, a significant association was limited to only females[102]. Our

study sample was younger than in both of these studies. We also adjusted for the effect of CRP,

an indicator of inflammation, which neither of the other studies did. In fact, our analysis suggests

that the relationship of area of VAT and cfPWV might be mediated through the inflammation

or/and insulin resistance as indicated by the altered significance level of associations of weight,

BMI, WC and area of VAT with cfPWV after CRP and insulin were added to the model.

In a Polish study, the total fat content (measured by bio-impedance) but not BMI was

positively correlated with arterial stiffness[212]. The Polish study used stiffness index, an estimate

of PWV, while we used cfPWV which is considered the 'gold standard' measurement of arterial

stiffness [206]. Ferreira et al found trunk fat mass (measured by DXA) associated with cfPWV,

however, their study differed from ours in several aspects including analytical approach[216]. Their

multivariate model may have been overly adjusted with highly correlated peripheral and trunk fat

together in a single model, although they mentioned the model was not disturbed by multi-

collinearity. Also, Ferreira et al's study sample were on average 14 yrs younger than ours, had

both male and female Caucasians, while we had only females representing two (CA and AA)

ethnic groups. In another study, with participants on average 19 years older than ours, the trunk

fat mass (measured by DXA) was associated with peripheral (carotid, brachial and femoral

arteries) but not with central (carotid-femoral) arterial stiffness as measured by carotid-femoral

transit time rather than cfPWV [213]. The HOORN study did not evaluate an association of the total

fat mass with arterial stiffness. All three studies, the Polish, the HOORN and Ferreira et al.'s used absolute value of trunk fat mass while we used proportion of fat in the trunk. We considered presenting association between absolute trunk fat mass and cfPWV, however, we decided the proportion of trunk fat relative to the total fat would be a better marker of abdominal fat distribution and would allow us to better evaluate the impact of trunk fat distribution on arterial stiffening.

Several mechanisms explaining the patho-physiological relationship between total and regional fat distribution and arterial stiffness have been suggested. Abdominal adiposity is associated with increased release of inflammatory markers, increased SBP, higher total and LDL cholesterol, increased insulin resistance, decreased HDL cholesterol, because of it's increased pro-lipolytic activity and higher ability to deliver free fatty acids and inflammatory markers directly to systemic circulation than other fat depots[26]. Increased adiposity may release higher amounts of circulatory pro-inflammatory markers including tumor-necrosis factor α (TNF- α), interleukin 6 (IL-6)[26,105,217] and elevated levels of inflammatory markers has been indicated to increase arterial stiffness[218-221] either by their effect on collagen/ elastin ratio or by impairing endothelial function[222] or via insulin resistance[221,223]. Similarly, the association of an increased aortic stiffness in the presence of impaired glucose metabolism and type-2 diabetes as suggested by previous studies[224] might indicate the deleterious role of glycemic imbalance on arterial stiffening[225]. Increased insulin level is suggested to contribute to arterial stiffness by promoting oxidative stress, endothelial dysfunction and vascular smooth muscle cell growth[213,226]. Thus, adiposity induced insulin resistance and increased availability of inflammatory markers may serve, at least partly, as a link between adiposity and arterial stiffness[9,227]. Our finding is

consistent with this concept, as indicated by the altered association of adiposity measures and cfPWV after allowing CRP and insulin in the model.

Additionally, bioavailability of leptin has been found to be positively correlated with adiposity and is suggested to reduce arterial distensibility[228]. In cell culture, leptin has been found to stimulate vascular smooth muscle cell proliferation and migration and thus may also influence vessel tone and remodeling[229]. Similarly, other peptides including adiponectin[230] and resistin[231] may explain, at least partly, the possible link between adiposity and vascular structure and function[213]. Since we did not have information on leptin, adiponectin and resistin, we were unable to explore their role on cfPWV in our study population.

Prior studies exploring the role of lipids on PWV have found minimal or inconsistent correlations[232-236]. In our univariate linear regression analysis, total cholesterol, LDL, HDL and triglyceride were not associated with cfPWV.

Given the cross sectional design of our study, we were unable to explore whether the observed associations are casual in nature. Since we are unaware of any statistical procedure to compare R^2 obtained from two or more regression models with different variables, we did not test whether the differences observed in R^2s using different measures of adiposity were statistically significant.

To conclude, our findings suggest that total amount of body fat has negative effect on arterial stiffness even after we account for other risk factors of arterial stiffness. Furthermore, anthropometric measures of adiposity appear to be as good as adiposity measures obtained by more sophisticated methodologies (CT and DXA) in explaining variances in arterial stiffness. Since anthropometric measurements require minimal training, are cost-effective, the instruments are easy to carry and maintain, does not involve radiation exposure, our findings support the use

of anthropometric in evaluating the role of adiposity on arterial stiffness. Also, the negative effect of total or regional body fat content on arterial stiffness may be mediated through a number of biological mechanisms associated with adiposity.

Baseline (BL)

259 w/ consent to participate in SWAN-Heart out of 453

Follow up (FU)

Carotid scan- 238
CT scan- 222

Carotid & CT scan- 222

Carotid scan- 212
CT scan- 185

Carotid & CT scan- 184

21 In FU but not in BL

222 (BL) + 21 (FU) = 243

236 matched to DXA

26 HRT/ Surgical menopause

210

1 different DXA machine

209 for Analysis
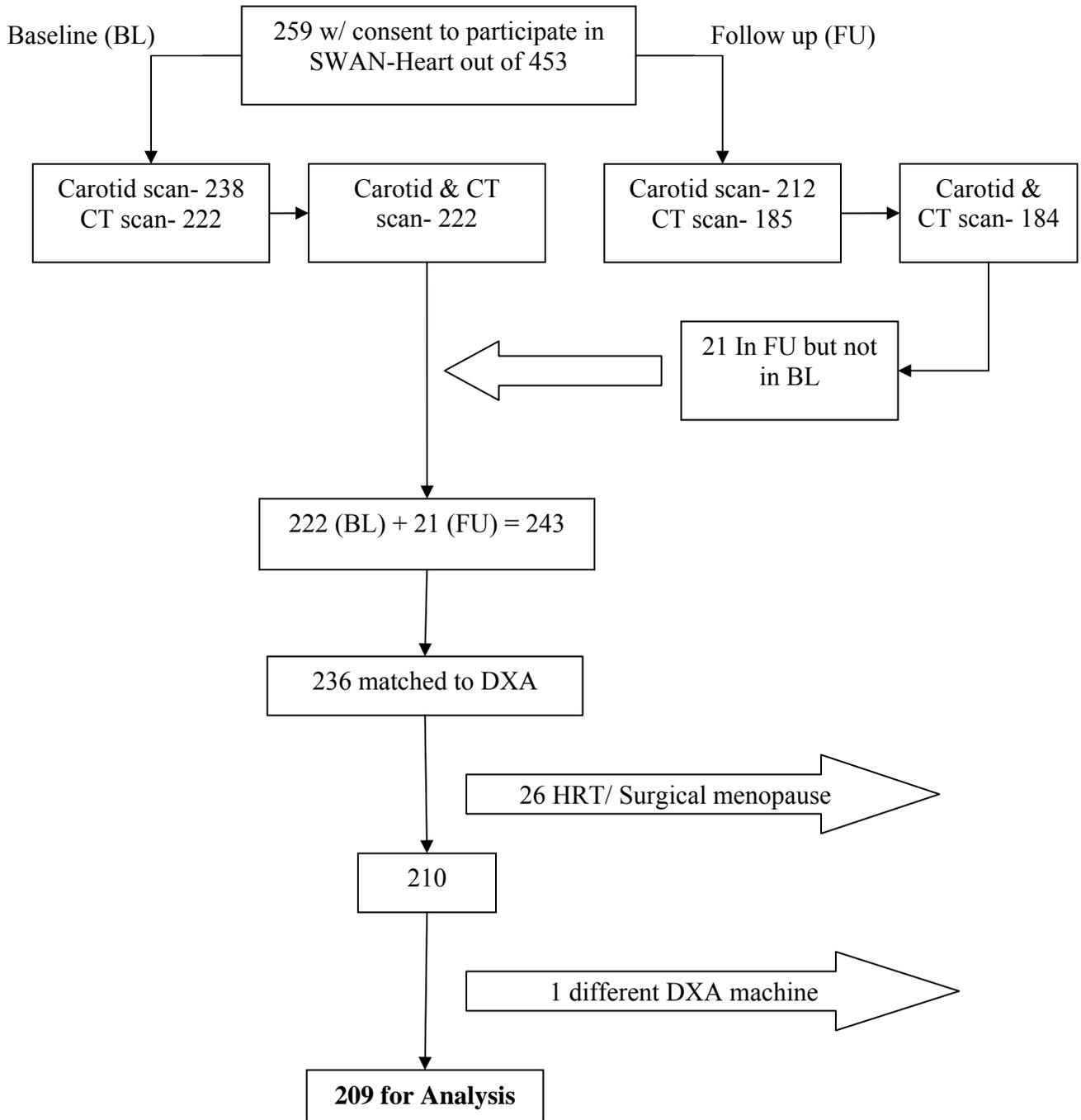
Figure 5-1. Flow chart of subject selection.

Table 5-1. Participant characteristics by tertile of pulse wave velocity[#]

Characteristics	Pulse Wave Velocity (m/s)				P-value
	Combined N= 209	1st Tertile (3.7 – 7.4) N= 67	2nd Tertile (7.4 – 9.1) N= 68	3rd Tertile (9.1 – 23.1) N= 68	
African American (%)	30.6	20.9	25.0	47.1	**<0.001**
Age (years)	50.3 ± 2.6	49.9 ± 2.7	50.5 ± 2.8	50.4 ± 2.5	0.248
Cholesterol (mg/dl)	209.4 ± 39.4	212.4 ± 43.5	206.5 ± 39.0	211.2 ± 35.3	0.875
LDL (mg/dl)	126.7 ± 35.1	128.9 ± 41.9	124.6 ± 31.4	128.3 ± 31.7	0.908
HDL (mg/dl)	57.6 ± 14.2	59.8 ± 13.8	56.7 ± 13.6	56.8 ± 15.1	0.224
Triglycerides (mg/dl)	128.1± 107.5	118.6± 64.8	136.3± 152.8	129.2 ± 86.6	0.663
Glucose (mg/dl)	89.5 ± 19.1	86.8 ± 10.9	88.4 ± 14.8	93.3 ± 28.1	0.091
Insulin (uIU/ml)	11.4 ± 7.6	9.8 ± 4.8	10.9 ± 7.7	12.8 ± 8.7	**0.039**
CRP (mg/ l)	4.0 ± 4.7	3.0 ± 4.1	3.6 ± 4.1	5.4 ± 5.7	**0.004**
DBP (mmHg)	72.6 ± 8.7	71.5 ± 7.9	70.9 ± 9.1	75.3 ± 8.7	**0.015**
SBP (mmHg)	114.5 ± 15.7	111.1 ± 14.5	112.0 ± 13.9	120.4 ± 17.2	**<0.001**
Pulse Rate (beats/ 30 s)	35.2 ± 4.2	35.1 ± 5.0	35.5 ± 3.7	35.0 ± 4.0	0.909
Pulse Pressure (mmHg)	41.9 ± 10.3	39.5 ± 3.7	41.0 ± 8.4	45.0 ± 11.1	**0.001**
Menopausal status (%)					
Later	43.4	47.8	33.8	48.5	0.917
Yes Smoking (%)	14.4	14.9	13.2	15.2	0.972
Education (%)					
>High school	77.6	70.2	85.3	77.3	0.288

[#]- Values are expressed as proportions (categorical) and mean ± standard deviation (continuous)

P-values are based on unadjusted ordinal logistic regression.
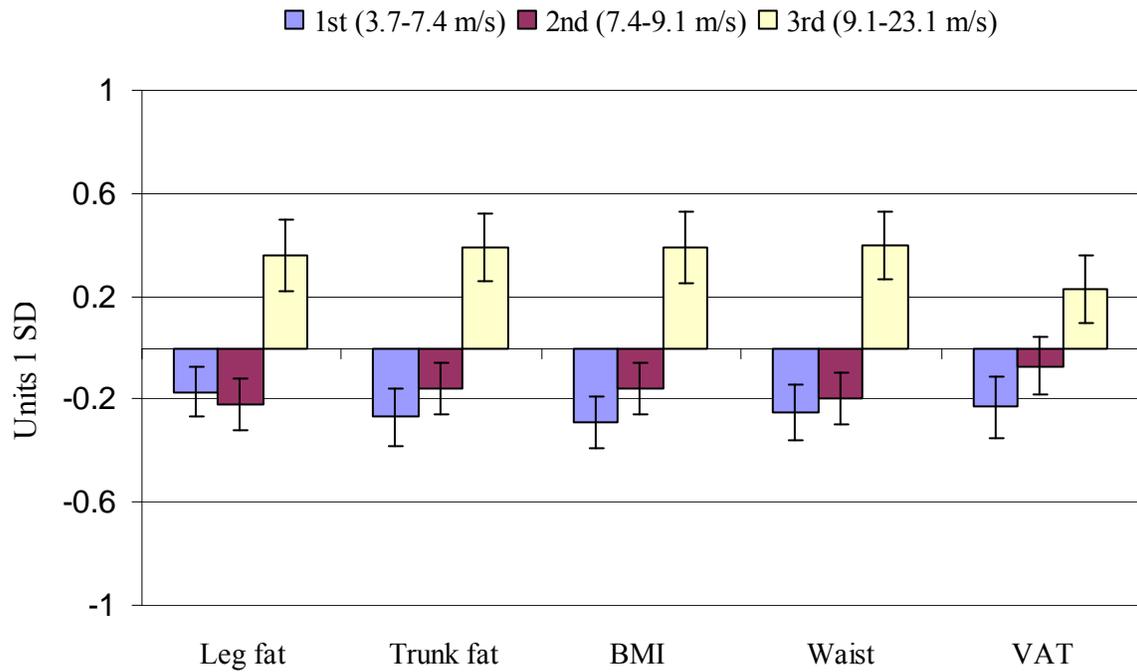
Figure 5-2. Adiposity measures by tertile of cfPWV

*- BMI, WC and trunk fat mass were significantly higher with increasing order of cfPWV tertile (all p-value <0.001). Leg fat (p= 0.001) and area of VAT (p= 0.005) were also significantly higher with increasing order of cfPWV tertile. P-values were based on ordinal logistic regression.

Note: The units are standardized to one standard deviation.

Table 5-2: Correlation of log cfPWV with predictors- (Spearman's)

Predictors	Log of cfPWV	
	Correlation coefficient	**p-value**
Age (years)	0.10	0.171
Weight (kg)	**0.28**	**<0.001**
BMI (kg/m^2)	**0.28**	**<0.001**
WC (cm)	**0.28**	**<0.001**
WHR	**0.20**	**0.004**
Total fat (%)	**0.28**	**<0.001**
Trunk fat (%)	**0.20**	**0.004**
Arm fat (%)	0.08	0.272
Leg fat (%)	**-0.21**	**0.002**
VAT (cm^2)	**0.23**	**<0.001**
SAT (cm^2)	**0.24**	**<0.001**
WAT (cm^2)	**0.25**	**<0.001**
Cholesterol (mg/dl)	0.008	0.913
LDL (mg/dl)	0.07	0.349
HDL (mg/dl)	**-0.16**	**0.029**
Insulin (uIU/ml)	**0.19**	**0.010**
Glucose (mg/dl)	0.09	0.229
C-RP (mg/l)	**0.24**	**<0.001**
DBP (mmHg)	**0.14**	**0.046**
SBP (mmHg)	**0.20**	**0.004**
Pulse Pressure (mmHg)	**0.22**	**0.002**
Estrogen	-0.01	0.930
SHBG	0.002	0.976
DHEAS	*-0.14*	*0.056*
FSH	-0.08	0.260

Table 5-3: Base multivariate linear regression models

Variables	Carotid femoral pulse wave velocity		
	Model R^2	β (SE)	p-value
Base variables			
Age (yrs)		0.010 (0.008)	0.223
African American	0.055	**0.130 (0.040)**	**0.002**
Later menopausal status		-0.007 (0.044)	0.883
Height (cm)		-0.0003 (0.003)	0.917
Adiposity measures[*]			
Weight (kg)	0.107	**0.004 (0.001)**	**0.001**
BMI (kg/m^2)[#]	0.109	**0.103 (0.003)**	**<0.001**
Waist (cm)	0.095	**0.004 (0.001)**	**0.004**
WHR	0.061	0.287 (0.270)	0.289
Total fat (%)	0.103	**0.007 (0.002)**	**0.002**
Trunk fat (%)[^]	0.091	0.004 (0.003)	0.145
Arm fat (%)[^]	0.091	0.011 (0.010)	0.264
Leg fat (%)[^]	0.131	-0.003 (0.002)	0.275
VAT (cm^2)	0.095	**0.0008 (0.0003)**	**0.004**
SAT (cm^2)	0.082	**0.0003 (0.0001)**	**0.018**
Whole fat (cm^2)	0.092	**0.0003 (0.0001)**	**0.006**

[*]- Adjusted for age, race, menopausal status and height.

[#] BMI- not adjusted for height

[^]- In addition to base variables, proportions of trunk fat, arm fat and leg fat were also adjusted for respective proportions lean mass.

β- parameter estimates; SE- standard error of parameter estimates.

Table 5-4: Fully adjusted multivariate linear regression models

Variables	Carotid femoral pulse wave velocity		
	Model R^2	β (SE)	p-value
Base variables			
Age		0.007 (0.009)	0.438
African American		**0.108 (0.047)**	**0.022**
Later menopausal status		- 0.026 (0.048)	0.586
SBP mmHg	0.087	0.001 (0.001)	0.313
Insulin uIU/ml		0.001 (0.003)	0.667
Height (cm)		0.002 (0.003)	0.525
CRP		0.007 (0.004)	0.126
Adiposity measures[*]			
Weight (kg)	0.102	**0.003 (0.002)**	*0.101*
BMI (kg/m^2)[#]	*0.101*	*0.008 (0.004)*	*0.086*
WHR	0.087	0.006 (0.317)	0.986
Waist (cm)	0.095	0.002 (0.002)	0.240
Total fat (%)	0.110	**0.006 (0.003)**	**0.042**
Trunk fat (%)[^]	0.099	0.003 (0.003)	0.454
Arm fat (%)[^]	0.149	0.013 (0.011)	0.249
Leg fat (%)[^]	0.143	- 0.002 (0.003)	0.580
VAT (cm^2)	*0.103*	*0.0006 (0.0004)*	*0.090*
SAT (cm^2)	0.093	0.0002 (0.0002)	0.299
Whole fat (cm^2)	0.098	0.0002 (0.0001)	0.161

[*]- Adjusted for age, race, height, menopausal status, SBP, insulin and CRP.

[#]- BMI not adjusted for height.

[^]- In addition to base variables, proportions of trunk fat, arm fat and leg fat were also adjusted for respective proportions lean mass.

β- parameter estimates; SE- standard error of parameter estimates.

Table 5-5: Change in variance before/after adjusting for SBP, insulin and CRP

Variables	Base Model[#] Model R^2	Expanded Model[*] Model R^2	% Change in R^2
Base model	0.055	0.087	58.2
Weight (kg)	0.107	0.102	-4.7
BMI (kg/m^2)[@]	0.109	0.101	-7.3
Waist (cm)	0.095	0.095	0.0
WHR	0.061	0.087	42.6
Total fat (%)	0.103	0.110	6.8
Trunk fat (%)[^]	0.091	0.099	8.8
Arm fat (%)[^]	0.091	0.149	63.7
Leg fat (%)[^]	0.131	0.0143	9.2
VAT (cm^2)	0.095	0.103	8.4
SAT (cm^2)	0.082	0.093	13.4
WAT (cm^2)	0.092	0.098	6.5

[#]- Base model adjusted for age, race, height and menopausal status.
[*]- Expanded model adjusted for age, race, height, menopausal status, SBP, insulin and CRP.
[@]- BMI not adjusted for height.
[^]- Regional proportions of fat were adjusted for respective proportions of lean mass in addition to base variables.

6.0   Overall Discussion

6.1 Summary of Findings

According to a CDC report published in 1999, the probability at birth of an American eventually dying from major CVD is 47% [69]. In general, females develop CVD ten to fifteen years later in life than males and CVD prevalence is higher among females after menopause (figure 2-6)[237]. Atherosclerosis has been suggested as the major cause of CVD [10,71] and has been positively associated with adiposity. Also, a significant change in total and regional adiposity with an overall gain in abdominal fat is frequently observed in women around or after menopause. However, research exploring the role of regional adiposity on S-CVD has been limited.

Therefore, in this dissertation, we investigated the role of fat partitioning on subclinical atherosclerosis, measured by carotid IMT and carotid AD, and arterial stiffness, measured by cfPWV. In chapter 3 we evaluated the role of total and regional adiposity on carotid IMT and AD among women in menopausal transition. In chapters 4 and 5, we examined the variance explained by different measures of adiposity on markers of atherosclerosis and markers of arterial stiffening. All three analyses were cross sectional and participants for all analyses were selected from a cohort of CA and AA women transitioning through menopause, enrolled in SWAN and SWAN-Heart studies.

Our results are in agreement with prior reports and extend them in several ways.

Available literature suggests that adiposity, mainly measured by anthropometric markers, BMI[87,142,155,157,159], WC[158], WHR[159], abdominal diameters[160] is positively associated with atherosclerosis. Anthropometric measures are considered indirect measures of adiposity as they lack the ability to distinguish fat from lean and bone mass. An increasing number of reports

suggest the differential role of regional adiposity on cardiovascular health [8,49,50,105,143,147] but none have thoroughly evaluated this relationship in women transitioning through menopause.

Our findings from the first analysis (chapter 3) suggest that adiposity is directly associated with AD among women during menopausal transition, which is thought be a critical time point in the process of natural aging. The findings further suggest that higher fat accrued in the abdominal area may have more detrimental effects on subclinical atherosclerosis than fat accrued in the lower extremities. Prior studies found positive association of BMI [155,159], WC [145] and IMT, however, other studies with more precise measures of fat such as VAT[148], fat mass[3] did not find significant association after controlling the effect of other covariates similar to ours' including age, race SBP, insulin, smoking status. A theory [93,95], already tested in animal models [96] that AD enlargement precedes IMT thickening may best explain the observed positive association of adiposity measures with AD but not with IMT. Alternatively, it is possible that our study population is in 'very early' stage of IMT thickening as indicated by mean IMT of 0.70 mm, a significant association of precise adiposity measures with IMT may not be apparent at this stage.

In second and third analyses (chapter 4 and 5), we evaluated the variances explained by 11 different measures of adiposity on atherosclerosis (measured as IMT and AD) and arterial stiffness (measured as cfPWV). We compared three methods namely; anthropometry, DXA and CT of measuring human adiposity in vivo. Prior studies that compared anthropometry against bio-impedance [158,172,180] or air displacement plethysmography [181] or CT [177]in relation to CVD or its risk factors.

Anthropometric measurements are most commonly used measures of adiposity in both clinical and research settings. Recent understandings of adipose tissue indicate that it is the fat

that has deleterious effect on disease outcomes rather than overall mass that includes muscle and bone mass. Traditionally used anthropometric measurements are now considered imprecise as they lack ability to differentiate fat from lean mass. Hence, newer technologies such as DXA and CT that are able to differentiate fat and lean mass are considered more precise and accurate in measuring total and regional distribution of fat and lean mass and to estimate their contribution to disease outcomes [18,26]. There is a substantial need for an 'ideal' marker of overall and regional adiposity would need to; a.) be accurate in measuring adiposity, b.) predict disease risk in general population, c.) be applicable and acceptable in large population studies as well as in clinical settings and d.) be precise resulting in small measurement error. Clear agreement on ideal marker of overall adiposity and regional adiposity is still far from reach. Technically, sophisticated newer methodologies have been indicated as the more accurate for analyzing body composition in human. However, superior ability of CT or DXA adiposity measures to explain variability in diseases over anthropometric measures is not clearly established. This clearly warrants further research in the field to identify an 'ideal' or 'close to ideal' measure of adiposity that can be interpreted in terms of disease outcomes.

In our second analysis, we found WC as good as more precise fat measures (VAT and proportion of trunk fat) in explaining variability in IMT and AD. Our findings suggest differential role of regional fat distribution; abdominal adiposity posing deleterious impact on AD and IMT and fat distribution in the lower extremities having some beneficial impact on both measures of outcome.

Among three studies[158,172,180] that compared adiposity measurements assessed by anthropometry against measurements of fat assessed by bio-impedance methodology, the largest one[172] (n= 12,608) found WC as a better predictor of risk factors of atherosclerosis. In the second

study[180], fat mass index (total fat mass/ squares of height) had a stronger association with CVD than WC, p-values for trend 0.03 vs <0.001. Similarly, the third study[158] found a stronger association of percent body fat (total fat/ weight) with CCA IMT than WC, p-values <0.0001 vs <0.05. Our findings are in agreement with the first study only. We used better measures of abdominal fat, proportion of trunk fat relative to total body fat, to compare against WC while the second study used fat index and the third study used percent fat. Furthermore, our findings may be more robust as the applicability of bio-impedance technology have been less satisfactory in real life settings due to the need of population specific equations for analysis and underlying assumptions of equal distribution of conductive material in different regions of human body [182-185].

Similar to our findings, a Japanese study comparing anthropometric measurements of adiposity against CT measurements did not find superior correlation of intra-abdominal or abdominal subcutaneous fat and carotid IMT when compared to WC or BMI among men [177]. Our study confirms Takami et. al's findings with greater confidence as we adjusted for more variables in the regression model than they did and validates their findings to American females in the menopausal transition. Bosy-Westphal et al. did not find the direct measure of adiposity assessed by air displacement plethysmgraphy methodology better than BMI or WC in explaining variances in several indicators of metabolic risk among individuals with high risk of metabolic syndromes [181]. We confirm their findings in apparently healthy women in menopausal transition with two direct methods (CT and DXA) of adiposity.

Our third analysis (chapter 5) examined the variability explained by eleven different measures of total or regional adiposity on arterial stiffness. Direct measures of overall or regional adiposity did not explain more variance on arterial stiffness than anthropometric measures after

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accounting for potential confounders or known risk factors of arterial stiffness. More importantly, in our analysis total body fat relative to total weight appeared to impact arterial stiffness negatively even after controlling for the effects of potential confounders and risk factors of arterial stiffness.

Prior studies have indicated that direct measures of adiposity might be better predictors of arterial stiffness than anthropometric measures [212,213,216]. Those studies used absolute values of either total or regional fat masses in contrast to the proportion of total and regional adiposity as we have used. In fact, we considered examining association between total or regional absolute fat masses and cfPWV, however, we decided the proportions of total fat relative to weight and proportions of trunk, arm and leg fat relative to total fat mass would be better measures of total and regional fat distribution. Our findings are in agreement with the concept that direct measures of adiposity might be better predictor of arterial stiffness than anthropometric measures as indicated by a positive association of proportion of total fat and cfPWV even after controlling for potential confounders/ covariates such as age, race, menopausal status, insulin, height and CRP.

In conclusion, our first and second analysis suggest that accruement of abdominal fat plays negative role in atherosclerosis than fat deposited in other body parts such as fat in the legs. The third analysis suggests that the direct measure of total adiposity may play more detrimental role in arterial stiffness than direct regional or indirect total or regional adiposity.

Furthermore, we found WC as good as direct measures of adiposity (VAT or proportion of trunk fat) obtained by using newer technologies such as CT or DXA with capabilities to distinguish fat from lean tissue in explaining variance in IMT or AD or cfPWV. WC, a measure of abdominal adiposity, requiring minimal training, being cost-effective and posing no risk of radiation exposure, may be more prominent in determining the effect of adiposity on vascular

health than VAT or proportion of trunk fat obtained by expensive and sophisticated technologies such as CT or DXA respectively.

Taken together, the findings from these three analyses support to the concept of differential role of excess adipose tissues accumulated in different anatomical regions on vascular health. Higher abdominal adiposity appeared to play more detrimental role on atherosclerosis than peripheral adiposity, while proportion of total body fat relative to body weight appeared to have deleterious effect on arterial stiffness. More importantly, our findings indicate that anthropometric measures are as good as sophisticated newer methods that have ability to differentiate fat from lean and bone tissues in explaining variances in vascular health.

6.2 Limitations

The SWAN and SWAN-Heart studies provided the unique opportunity to investigate the relationship of total and regional adiposity on S-CVD among women in menopausal transition. However, there are some limitations applicable to all three papers that should be considered when interpreting the findings from this dissertation.

The study cohort for all three papers consisted CA and AA women in the menopausal transition. Thus, the findings from this dissertation may not be generalizable to women from other ethnic groups and/ or women in other menopausal status. Although, prior literature has indicated that S-CVD and the body fat distribution may vary by ethnic groups, we were unable to test if our findings were different for CA and AA because of lack of statistical power.

More importantly, SWAN is a study of women going through menopause and therefore only included women. Hence three analyses in this dissertation were limited to women and the findings may not be generalizable to men.

Since all three papers were designed as cross-sectional, the association of fat distribution and vascular health may not be interpreted as casual. Comparisons of variances explained by different measures of adiposity on IMT, AD and cfPWV were solely visual as we are not aware of any statistical method that allows to compare different R^2 obtained from different linear regression models with different variables. Hence, we were unable to test if the observed differences in variances were statistically meaningful or not. The altered association of adiposity measures and measures of S-CVD after adjusting for traditional risk factors might be due to relatively small sample sizes or due to other biological mechanisms involved in between adiposity and S-CVD. Also, our ability of detect a significant association of some of the adiposity measures and IMT might have been limited because the study population is relatively healthy and thus the associations are not apparent at this stage. Since our analyses involved a number of multiple comparisons, it may have increased type I errors slightly.

6.3   Public Health Significance

Obesity and CVD are major public health problems. Two in three Americans are either overweight or obese. Although our understanding of CVD and obesity has been improved substantially in recent past, CVD still remains the major killer of Americans while obesity is increasing in prevalence. In 2003-2004, 32.2% US adults (20 years of age or older) were obese [2]. Recent findings suggest that obesity has been increasing more rapidly than overweight. Proportion of overweight American, aged 20-74 years has increased by more than 45% in 1999-2002 compared to 1960-1962, while for the same period and age group, obesity rate increased by more than 133% [11]. Apart from its negative health outcomes, obesity also induces enormous

amount of health care expenditure. In 1998 direct cost attributed to obesity (not overweight) was 70 billions US dollars in the US which was 7% of the US health care expenditure for the year [15].

In the US, one of the major goal of healthy people 2010 is to substantially increase life expectancy in the next 10 years [238] which is likely to translate into bigger burden of chronic disease including CVD and obesity as well as economic burden to the state. The continual increase in obesity induced disease burden call for better and more aggressive preventive strategies on the public health level. Prevention of disease, death and disabilities related to CVD and obesity requires early recognition and diagnosis in order for appropriate clinical, lifestyle or behavioral intervention. In general, it is understood that increased adiposity increases the risk of CVD through a number of indirect pathways. However, the direct relationship of increased adiposity and CVD has yet to be established. Elucidating the inter-relationship of regional adiposity and CVD may help to understand the mechanistic pathways amenable to lifestyle, behavioral and pharmacological intervention in order to delay or prevent future CVD events, particularly for people with prevalent obesity. Hence, understanding the biological mechanisms of obesity and CVD is of a crucial importance.

Furthermore, findings from chapters 4 and 5, waist circumference being as good as VAT or proportion of trunk fat in explaining variance in IMT, AD and cfPWV are particularly important for designing similar future studies. Using waist circumference over other adiposity measures obtained from expensive and sophisticated technologies will be cost-effective, poses no radiation risk to the subject, needs minimal trainings and is easily replicable. Also, using WC as a screening tool to identify people at increased risk for CVD in subclinical level might be an advantage in population level to call for an appropriate intervention for primary, secondary or tertiary prevention of negative health outcomes.

6.4 Future Research

Both obesity and CVD are the result of several factors including genetic, environmental and behavioral. While many independent studies have indicated a number of biological link between obesity and CVD, the agreement on 'ideal' or close to ideal measure of total and regional body fat distribution is still an elusive goal. It is therefore important to carry out epidemiologic longitudinal studies that may lead to the general agreement on the best measure of fat distribution. Also, longitudinal investigation of change in regional fat distribution might help us to understand the fat re-partition associated with natural aging and its relative importance in terms of disease outcomes. Such studies need to be carried out in populations with diverse ethnic background, wide age range incorporating both genders. Since longitudinal epidemiological studies of fat distribution might be very expensive and burdensome to the study subjects, embedding such studies into the studies with other primary outcomes may serve as a better option.

Investigating if WC is as good as VAT or proportion of trunk fat, in other populations, particularly in multi-ethnic populations, might be helpful in identifying the best abdominal adiposity measure. Also, future studies should be directed to test these findings among men and in population with different age groups. Furthermore, if such future studies can confirm our results, WC being as good as VAT or proportion of trunk fat in explaining variances in vascular health, in other populations that may help to maximize the health care resources as DXA and CT methodologies are very expensive as compared to anthropometric method. Additionally, our findings call for longitudinal studies in future to test if AD enlargement precedes IMT thickening, to identify practical cut-off value for IMT that has clinical significance in population level. Identifying clinically significant cut-off values of IMT, one of the most widely used

marker of atherosclerosis, might serve as an important tool for secondary or tertiary prevention of atherosclerosis.

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